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FOREWORD

THIS ANNIVERSARY NUMBER was intended to include articles from investigators who have long been associated with the first Editor of ENDOCRINOLOGY, either through participation in the activities of the Association or in connection with the Journal. The intention has been fulfilled to a high degree.

The Committee expresses its gratitude to contributors for their sincere and effective cooperation. It is regretted that several papers which had been promised could not be completed in time for inclusion in this number, due to circumstances quite beyond the control of the authors.

COMMITTEE FOR THE ANNIVERSARY NUMBER
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ROY GRAHAM HOSKINS

AN APPRECIATION

IN THE DECADES either side of the new century the University of Kansas had some remarkable teachers and also some remarkable students. Perhaps there was mutual stimulation. Among the teachers were McClung, expert in chromosomes of grasshoppers, who was abundantly supplied with research material by a bounteous nature; Williston, respected as an intellectual giant because famous for investigations in two fields—entomology and paleontology; Barber, who showed how to pick up a single bacterium and manipulate it; E. C. Franklin, well known for his extension of the newer ideas and methods of physical chemistry; Dyche, zoölogist, taxidermist and arctic explorer; Blake, physicist and inventor; and others in non-scientific fields. Later these men were scattered—to Pennsylvania, Chicago, the Rockefeller Foundation, Stanford—but as young and eager workers in science they labored together in Lawrence and surrounded themselves with groups of enthusiastic disciples.

Of the student group many turned to scientific careers. McCollum in nutrition at Hopkins, Adams in zoölogy at Illinois, Sellards in tropical medicine at Harvard, Hartman in physiology at Ohio State, Scammon in anatomy at Minnesota, Meek in physiology at Wisconsin, Burrows in tissue-culture and cancer research, Hixon and Weidlein in chemical engineering—all were started at Kansas in the early years of this century.

Among these students was Roy Graham Hoskins. His youthful experiences had been those of the oldest of six children in a pioneering family, knowing life in the primitive conditions of Nebraska and Kansas prairies, where sod huts were homes and men and boys toiled hard together for clothing, food and shelter. The year after he finished his high school education, at the age of 19, he was appointed both Principal and sole teacher in another high school—the two said to have functioned quite harmoniously. The University of Kansas gave him the baccalaureate degree in 1905, and the Master's degree in 1906. During the next two years he taught; first as Fellow in Zoölogy in the University, second, as Science Teacher in the Chanute (Kansas) High School. An alert mind, a pleasant disposition and skillful fingers were noted as characteristics by his collaborators in the Zoölogical Department.

Apparently collecting-expeditions were a favorite type of scientific activity in Kansas University about the turn of the century. Young Hoskins engaged in two of them during summers while an undergraduate, one in western Kansas, the other in eastern Oregon. Report has it that in the field his optimism, his enthusiasm and his buoyancy of spirit did much to maintain a cheerful morale in the whole group. His inspirational singing appears to have been accepted without adverse comment, for he had trained himself, and incidentally had helped pay his college expenses, by chanting Latin hymns in Sunday services. His biscuits, baked on the expedition of 1904, have not received so much praise—a friend declares that they were used to throw at the jack rabbits. A whimsical humor frequently slipped into his conversation and comments; in registering purchases he once playfully suggested that hard candy might properly be set down as axle grease, for did it not lessen friction—among the explorers?

It was while in the Department of Zoölogy that Hoskins learned that his interests were better satisfied by the functional rather than by the structural aspects of bio-

logical phenomena. Even on one of the collecting-expeditions he was not bothered by the leaf hoppers which rained down from the willows onto the lunch table—he became too much entranced by the ways they met the problem of adjusting themselves to strange surroundings. When Professor McClung clearly perceived that the Teaching Fellow in his Department was more deeply concerned with physiology than morphology, he wisely advised him to turn to the possibilities of a career as a physiologist.

It was in the autumn of 1908 that Roy Hoskins came to the Physiological Laboratory of the Harvard Medical School. Just why he chose to do so has never been clarified. Up to that time my investigations had been concerned with the functions of the digestive tract. He wished to work on glands of internal secretions. About them I had only a literary acquaintance. Furthermore, he already had in mind a research program; he planned to determine whether removal of glands from pregnant mothers or feeding pregnant mothers gland extracts would affect glandular development in the offspring. (Later others worked on that idea!) The Harvard Faculty of Arts and Sciences had just established a Division of Medical Sciences that administered award of the Ph.D. degree. The achieving of that degree was the goal. I had not had experience in preparing a student for the degree, I knew nothing first-hand about the ductless glands—and in the circumstances the new candidate began my education in two important directions. When he received the doctorate in 1910 I had learned something about developing a disciple, and also had become so much interested in endocrine physiology that de la Paz and I undertook a research on the effects of emotional excitement on secretion from the adrenal medulla—an enterprise lying in the intermediate zone between gastro-enterology and endocrinology.

Immediately after finishing his experience at Harvard, in 1910, Dr. Hoskins was appointed Professor of Physiology at the Starling-Ohio Medical College at Columbus. There he devised the use of a segment of rabbit intestine as an indicator of secreted adrenaline—an indicator later employed extensively for many years for quantitative studies by Stewart and Rogoff in Cleveland and by Satake and his numerous collaborators in Sendai, Japan. At the end of a year of teaching in Ohio my first Doctor returned to Boston and we worked happily together in the summer vacation, securing evidence that asphyxia and sensory stimulation, which induce discharge of sympathetic impulses, likewise discharge adrenaline from the adrenal glands.

The three years at the Starling-Ohio School (1910–1913) disclosed a persistent drive for research and a deep interest in teaching. Three young men (McClure, Means and McPeek) were attracted to researches in collaboration with the Professor. The commonly held idea that the height of blood pressure was maintained by medulliadrenal secretion they badly damaged by tying off the adrenal glands and proving that the pressure was not lowered, and also by demonstrating that an amount of adrenaline which would not raise the pressure would definitely check intestinal peristalsis—a decisive and illuminating *reductio ad absurdum*. Meanwhile there appeared indications of an interest in the action of pituitrin. Papers on the correlation of clinical teaching and physiology, and on ways of encouraging medical research, displayed aspects of collateral thinking which the responsibilities of an academic position had stimulated.

The enterprising scholarship manifested at Starling-Ohio was the occasion for a call from Northwestern University Medical School in Chicago. There he went in 1913 as Associate Professor of Physiology—a title which he held until 1916 when he was made Professor and Head of the Department. Here is testimony regarding the influence of Dr. Hoskins in his new surroundings.

His Department became one of the most productive in the Medical School. This was advantageous not only for Physiology; it set a pace for other departments to follow. The result was increased interdepartmental interest in several problems which, while worked out independently, bought a common viewpoint which was reflected in the teaching. The student body realized more clearly the relation of certain fundamental medical subjects one to another, and, more important still, their relation to clinical medicine.

One of the pleasant episodes which heightened the week during the years Dr. Hoskins was a member of the Faculty was the Tuesday luncheon. The members of the laboratory branches attended and as many of the clinical staff as could take time to attend. Dr. Hoskins was a leading figure in these meetings. He offered space in his laboratory for the gathering, he delegated his technician to attend to the catering, and usually he was the one to lead the discussion. Not infrequently he would bring forth an important piece of work which had recently been reported and thus would keep some of the newest literature of medical interest before the group.

Again we behold the picture of a stimulating personality who affected in an admirable manner the habits and customs of a body of men by an exuberant initiative. This infectious enthusiasm was evidenced also by success in drawing into collaborative research at least a half-dozen young disciples (Wheelon, Lovelette, Rowley, Rosser, Gunning and Berry) who participated in a goodly number of the 23 articles published during the four years of his stay at Northwestern. In addition he worked with S. W. Ranson on the vasomotor action of nicotine. Interest in endocrinolgy was clearly broadening. Although 13 of the 23 articles were concerned with the adrenal glands and adrenaline, there were two on pituitrin and one each on the pancreas, the parathyroids and the ovaries. Among those on adrenaline were important reports of its effects on the distribution of blood in the body, especially its opposite action on the blood vessels of the skin and of muscles.

In 1917 the United States was drawn into World War I. Just as Dr. Hoskins was reaping the fruits of his enthusiastic teaching and his leadership in research he was called to a responsible position in the Section of Food and Nutrition in the Sanitary Corps of the United States Army. His departure from the Northwestern Medical School is said to have left "two vacancies,—one which had been filled by Hoskins the man and friend, the other by Hoskins the scientist." Although he was on leave of absence in the years 1917 to 1919, he did not return to Chicago at the close of his services to the Government. Instead he undertook studies at the Johns Hopkins Medical School, studies which resulted in his receiving, in 1920, the M.D. degree.

During the interim between academic activities, while he was in the U. S. Sanitary Corps, he acquired a new set of interests. He studied and wrote on the management and dietaries of military hospitals and on the way food should be served. Although not much is told about these years and their content, Dr. Hoskins' labors were evidently approved, for he was advanced, during his brief term, from the rank of Captain to that of Major.

The experience in Washington and Baltimore included a year's appointment as Associate in Physiology at Johns Hopkins. It also had the effect of providing a better acquaintance with clinical phenomena of endocrine disorders. Still another consequence was the establishing of a friendship with Dr. L. F. Barker. Dr. Barker had become impressed with the need for a comprehensive treatise on metabolism and internal secretions—a treatise which should "bring together, as far as possible, in reasonable compass, convenient for perusal and reference, all the essential facts . . . in health and disease." He assumed the editorship and selected as associate editors Dr. Hoskins for the sections on endocrinology and Dr. Herman O. Mosenthal for the sections on metabolism and its disturbances. The four volumes of text, each more than

900 pages long, and the fifth volume of index and references, nearly as long, were published in 1922. They represent a prodigious amount of labor. Dr. Hoskins himself contributed the introductory chapter, and also the chapters on the relations of the suprarenal glands to the circulation, on the physiology and pathology of the thymus and on the interrelations of the endocrine organs.

Service in Washington, working for the M.D. degree, teaching, and helping to edit a huge treatise were not the only activities of this peroid. In 1917 an Association for the Study of Internal Secretions had been organized and the journal, ENDOCRINOLOGY, had been started, with Dr. Hoskins as the Editor. It began as a quarterly publication. The routine necessitous duties of editing were, therefore, an additional burden being borne by Roy Hoskins during the heavily charged years in Washington and Baltimore.

Some of this burden he took along to Ohio when, in 1920, he returned to Columbus and to tasks of organization. The Starling-Ohio Medical School had been fully merged with the State University, becoming the Ohio State University College of Medicine. Dr. Hoskins again accepted appointment as Professor of Physiology and Head of the Department. He correlated the teaching of physiology in the professional and academic departments, supervised the arrangement of the physiology laboratories in a new building put up for the medical and dental colleges, developed seminar courses, taught classes, and outlined and directed research. Again, in spite of the load which was carried, inspirational leadership was revealed. More young men (Albritton, Tuttle, Hunter, Gans, Lee, Durant, Hitchcock, Powelson, Boukalik) were attracted to the laboratory and to investigative activities, more studies were reported (on the adrenals, pineal body and gonads), and a series was begun on conditions affecting bodily vigor, particularly removal of endocrine organs or administration of endocrine products.

The year 1927 saw another sharp change in Dr. Hoskins' activities and prospects. Through the interests and counsel of Dr. Allan Winter Rowe, Mrs. Stanley McCormick had become deeply impressed by the possible relations of the ductless glands to disturbances of cerebral functions, and had proposed the establishment of a Foundation for Neuro-Endocrine Research. A primary problem was that of finding a director—a man with medical training who had a broad acquaintance with the facts of endocrinology and who was gifted with imagination, good critical judgment and ability to work effectively with collaborators. After careful consideration of possible candidates Dr. Hoskins was asked to assume responsibility for the new enterprise. He moved to Boston (bringing with him one of his Ohio graduates, M. O. Lee), set up laboratory rooms and an office in the Harvard Physiology Building—a return to early associations—and began coöperation with the Massachusetts Department of Mental Disease at the Worcester State Hospital.

The subject selected for study, out of the broad range of possible endocrine factors in the psychoses, was the schizophrenic psychosis. Direction of this comprehensive research required acquaintance with the methods of physicists, chemists, psychologists, experts in biometrics, clinical psychiatrists, physiologists, besides an understanding of the problems of hospital administration. This was a challenge which Dr. Hoskins, disciplined to meet and overcome obstacles, accepted with confidence. For fifteen years he and his associates have applied their skill and intelligence to an accumulation of observed facts in the lives and conduct of victims of schizophrenia that have been unmatched in the history of psychiatry. It should be emphasized that in this undertaking a novel feature has been the installation in a custodial hospital of a group of coöperative scientific investigators. The scheme has deeply stirred the in-

terests of the local staff in problems of psychiatry, it has attracted attention and support from other sources than those of the original Foundation, and it has had favorable effects which have resulted in annual grants from the State, on the conviction that money spent for research lessens the necessity of spending money for new buildings. The demonstration that a body of research experts can be introduced into an 'asylum' and can conduct scientific studies of patients which yield valuable information, besides transforming the attitude of the staff, constituted a significant advance in the care of 'asylum' patients. The plan was proved economical because no new buildings were required, rooms could be set aside for laboratories, and needed heat, light and power were incidental in the operations of a large institution.

During the fifteen years, while part-time at Worcester directing clinical investigation, and part-time in Boston engaged in experiments on laboratory animals, Dr. Hoskins has published more than three-score articles, besides writing an elaborate annual report recounting past accomplishments and outlining future plans. No fewer than twenty-three different persons have collaborated with him in the researches which have been reported. Of course, many more papers have been published individually by members of the groups whose work he has stimulated. Schizophrenia in relation to the thyroid, to the suprarenal cortex, to insulin, to gonads—all have been considered, besides special examination of basal metabolism, cardiovascular conditions, galactose-tolerance, weight changes and numerous other features. The search for the essential nature of the disorder has been long and arduous. An immense body of observed characteristics, bodily and mental, has been accumulated. The effort is still in progress and the eager and optimistic spirit of the Director is not daunted.

As previously mentioned, Dr. Hoskins began in 1917 to edit ENDOCRINOLOGY. In the second year of the venture the conditions were considerably better than in the first year "due to Dr. Hoskins and his assistants in their untiring work," according to the official record. By 1921 the Association for the Study of Internal Secretion and additional subscribers had brought the subscriptions up to more than 2000 and in January of that year the Journal was changed from a quarterly to a bimonthly issue. About this time, it will be recalled, Dr. Hoskins was studying for his medical degree at Johns Hopkins and serving as co-editor of "Endocrinology and Metabolism." A close friend in those years testifies to amazement at his "enormous capacity for effective work," especially when hard days, spent on routine labors, were followed by attention to the problems of ENDOCRINOLOGY until the small hours of the morning. In 1925 the Editor wished to resign, but the Council of the Association unanimously endorsed his services and besought him to continue to serve. He consented. The next year he was honored by election to the presidency of the organization.

The regular recurrence of duties to ENDOCRINOLOGY, which became a monthly periodical in 1938, pinned the Editor closely to his desk. Whether in sickness or in health, in freedom or in bondage to other obligations, the editor of a periodical publication is compelled by persistent obligations. As sure as the days pass the requirements of the job inexorably appear. Such was the task performed by Dr. Hoskins, cheerfully and well, in addition to many other demands on his time and energy, for 23 years, from 1917 to 1940. There is no doubt that the Journal has had a large influence in arousing attention to the significance of internal secretions for physiology and pathology, not only in this but also in other countries. And there is no doubt that by the selection of papers and by lectures and other influential acts the Editor served the highest aims of the medical profession by helping to hold clinical endocrinology, especially in the early years, close to a sound observational basis. At a time when this promising new realm in biology and medicine was threatened with

ridicule and scorn because of the wild surmises and fantastic claims of brethern whose imagination outran both fact and reason, the well considered views, and occasionally the sharp criticism, expressed frankly and boldly by Dr. Hoskins, were effective in evoking unwholesome tendencies. For that outstanding merit he deserves the highest praise.

Long experience in editing has fixed in Dr. Hoskins a sensitiveness to avoidance of redundant phrasing that shows in his own writing. It is clear, concise and strictly pertinent to the subject in hand. When such style is used for expressing the ideas of a man who sees events in unconventional patterns, the result is likely to bring titillating and impressive surprises. Many examples might be cited. Thus, when it was claimed that because adrenaline, used as a drug, raises blood pressure and the inference is drawn that therefore low pressure must be due to 'hypoadrenalism,' the shrewd comment that by the same reasoning constipation might be regarded as 'hypocascarism' made the point both definite and memorable. The volume, "The Tides of Life" (1933), expounding endocrinology for laymen in scientific rather than in fanciful terms, well illustrates the skill in simple, direct and entertaining exposition that flows from a natural freshness of spirit and a long and exacting discipline in use of our language.

In 1936 the National Research Council was asked by one of the larger Foundations for a report on the opportunities for useful employment of funds for promoting knowledge of endocrinology. The formulation of the report required a brief survey of significant progress already achieved and an outline of future possible advances in the field, a statement which would be both factual and imaginatively attractive. The long experience of Dr. Hoskins as editor of ENDOCRINOLOGY, as co-editor of the volumes "Endocrinology and Metabolism," as director of neuro-endocrine research in Boston and Worcester, and as author of "The Tides of Life," gave him a unique grasp of the whole range of interests which had to be presented. He proved to be an especially useful member of the Committee appointed to prepare the report. As a consequence of representations of the importance of promoting research in endocrinology, for both scientific and practical reasons, the Foundation has for the past five years placed at the disposal of the National Research Council generous funds for the support of studies in the realm of internal secretions—funds which have been allocated to active investigators in the United States and Canada by the Committee on Research in Endocrinology. In the functioning of this Committee Dr. Hoskins has played a very helpful rôle because of his wide knowledge and his wise counsel.

The life of Dr. Hoskins has not been free from tragedy and sorrow. Death has repeatedly taken from him persons who were near and dear. A younger brother, of whom he was especially proud, because of his achievement and high promise as a worker in science, died all too soon; his going left a deep sense of grievous loss. These occasions for sadness, however, did not alter the buoyant and cheery nature which was known to the outer world. And as time heals wounds of the body so likewise it heals wounds of the spirit. If one wishes to see Dr. Hoskins in his merriest mood one should visit him in his summer home on an old New Hempshire farm. With its rocky skeleton showing here and there it is indescribably different from the Kansas plains of his boyhood. But it invites the varieties of activity which the pioneering years called forth—planting and tending a garden and in due season gathering its fruits, carpentering, painting, wall-papering, tramping with his wife and children and enjoying in simple directness the beauty and charm which nature provides.

Is not pioneering the key to the life of Roy Hoskins? He developed in a family of pioneers. His abilities—his independence, his self-reliance, his resourcefulness, his

readiness for new ventures—were all drawn forth and strengthened by the hard, exacting years of his boyhood and youth. And the tests of the later years have on occasion after occasion drawn forth still more of these same characteristics. He brought with him his own ideas as he started in endocrinology; he assumed the responsibilities of a professorship as soon as he had his doctorate; without guidance or previous experiences he undertook editing the first periodical ever devoted to endocrine interests; he led in developing and directing a large research group in a state hospital, thus setting an important precedent—all evidences of an original and enterprising mind. He has seen attention to endocrinology develop from small beginnings, when he was first attracted to its problems, until now researches in the field are receiving generous financial support; he has watched the modest little quarterly, ENDOCRINOLOGY, develop into a handsome monthly publication receiving the reports of researches of numerous investigators in this and other lands; for years he has exerted his influence effectively in encouraging and restricting the medical uses of endocrine knowledge within sane and reasonable limits so that recently a new JOURNAL OF CLINICAL ENDOCRINOLOGY has been established. The pioneer has seen the wilderness change towards order and refinement and respectability—and can look back with satisfaction on the rôle he has played in the transformation.

WALTER BRADFORD CANNON

THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS. ITS PAST: ITS FUTURE

F. M. POTTENGER*
MONROVIA, CALIFORNIA

CERTAIN THERAPEUTIC PRODUCTS are particularly suited to the game of racketeers. Such are vitamins sold over the counter, advertised over the radio and in the newspapers, and allegedly added to various foodstuffs. The products of the endocrine glands are also particularly adapted to exploitation. When first they were brought to the attention of the public there was so much discussion and so much written about them in the press that demands were made for their therapeutic use long before products which were of value could be discovered.

It had been evident for some years that the internal secretions were sooner or later to become the object of intensive research. From the time Addison ascribed to the disease which bears his name a relationship to the adrenal gland, and diabetes was associated with disease of the pancreas, and myxedema was classed as being the result of a deficiency in the secretion of the thyroid, it was clear that the study of such diseases could not be delayed beyond the time when a suitable approach could be determined.

It seemed evident in the decade after 1910 that this time had apparently come. Moreover, it was desirable that such research be directed so that the public should be protected from charlatans who profit by making fraudulent claims. To this end the Association for the Study of Internal Secretions was pledged. It was organized in 1916. It developed and established high and what seemed almost impossible standards for endocrine products.

This Association was the first international society formed for the study of endocrinology. Fortunately for the organization and for the public, this association was controlled by medical men of high ideals. Among those who were especially active in its early guidance were Doctors Bainbridge, Barker, Cannon, Crile, Cushing, Daland, Dana, DeBuys, Goetsch, Hoskins, Hoxie, Kendall, Novak, Potts, Plummer, Sajous, and Timme. Dr. Hoskins, Editor of the Journal, ENDOCRINOLOGY, had the support of these men, insuring that the subject would not be exploited as a pseudoscientific affair.

As the years passed the Association secured the interest and gathered into its fold practically every scientific investigator in the field, and now has a galaxy of research workers rivaling those who gave bacteriology to medicine.

It was my pleasure to serve as Secretary of the organization during its formative years, and it was my honor to serve as its President after it had become well-established. At first it was a discouraging task to keep the Association going, because of the disappointment at the lack of effective products; in fact, when the Association was established there were only two dependable endocrine products available—thyroid gland substance and adrenalin.

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* Secretary-Treasurer of the Association for the Study of Internal Secretions, 1918–1935; President, 1935–1937.

It was impossible for endocrinological facts to be developed as fast as the public demanded, and this slowness came near wrecking our effort. Nevertheless, by appealing to the loyalty of the members of the medical profession and to their confidence in the future, and by working sympathetically with the manufacturers of biologic products, we were able to secure a coöperation to the end that the development of endocrinology was held on a high plane, and the public was protected.

Our problem was particularly difficult because the existence of the Association depended upon the support of clinicians who were being besought by their patients for help which they were unable to give. Accordingly there was more or less disappointment expressed by our members, not only by complaint but too often by resignation. This made the financing of the early years exceedingly difficult. It was necessary to make continuous appeals to the friends of our most interested members in order to fill our annually depleted ranks and keep the Association alive.

However, the leaders never lost faith that the future of the Association, could it survive, would be a glorious one; but they knew equally well that its future was precarious because of the constant uncertainty of survival. In order to meet deficits there were a few members who contributed generously, and finally, through the agency of Mrs. Stanley McCormick, a yearly grant was secured and was continued sufficiently long to put the organization on a sound business basis. This beneficence, the steadfastness of the inner circle, and Dr. Hoskins' persistent effort held the Association together until a sufficient number of facts regarding endocrinology were discovered to make it a real power in medicine. Nor must the faithful work of Mr. P. C. Mason as Assistant Secretary and Business Manager during these years be underestimated.

Dr. Hoskins, and later Dr. Lee, made ENDOCRINOLOGY a Journal of which medicine can well be proud. Today, after twenty-five years, we have a virile organization, publishing two journals, the one for reports from the laboratory, the other for clinical papers.

The Association has now made history, and its members may desire to know something of its past. So I will detail some of the incidents which marked its beginning and which, in case of need, may serve as its birth certificate.

The Association was founded by a group of men called together by Dr. Henry R. Harrower, of Glendale, California, at the Hotel Tuller in Detroit, June, 1916, at the time of the meeting of the American Medical Association. He had interviewed many men in different cities of the United States and gave it as his opinion that a sufficient number were interested in endocrinology to warrant the establishment of an association for its study. A Committee on Organization consisting of Doctors Lewellys F. Barker, Judson Daland, L. R. DeBuys, Emil Goetsch, Henry R. Harrower, George H. Hoxie, and John B. Potts, was appointed to report in New York City, in June, 1917, during the week of the next meeting of the American Medical Association. Dr. Harrower presented the names of three hundred charter members. Many of these, however failed to become active when the organization was completed.

A program was given at the New York Children's Hospital and School, at Randall's Island, which was considered the first under the auspices of our Association. Three papers of a total of thirteen were devoted to endocrinology as follows.

The influence of endocrine disturbances in the causation of feeblemindedness, with a presentation of several cases showing the influence of treatment. *Dr. Walter Timme, New York.*
Polyglandular disorders with relation to epilepsy. *Dr. Charles L. Dana, New York.*
Some cases of internal glandular therapy among the mentally retarded children. *Dr. Mary Sutton Macy, New York.*

A business meeting was held in the Hotel Manhattan, in New York City on June 4, 1917, at which the permanent organization was formed. Dr. E. Eliot Harris, New York City, was elected Chairman *pro tem*, and Dr. George H. Hoxie, Secretary *pro tem* of the meeting. Dr. William Seaman Bainbridge, of New York City, spoke of the work of Dr. Eugene Hertoghe, of Antwerp, Belgium, whose work in endocrinology had done much toward furthering experimental and clinical research in this subject. Dr. Hertoghe, who was financially independent, had intended to devote both his finances and scientific ability to research in this important field when Belgium was overrun by German soldiers, which caused him to abandon his plan. Dr. Hertoghe had expressed the hope that an international association for the study of internal secretions might be formed.

Dr. Lewellys F. Barker outlined the aims and objects of the Association, saying in part: "We are hoping that the Association for the Study of Internal Secretions will bring together members of the profession who are contributing to the progress of the study of the endocrine glands, whether that study be physiological, chemical, histological, or what not. We need to have the work brought together. We hope that the bulletin of the Association, ENDOCRINOLOGY, will bring together all the work bearing upon the study of internal secretions. Above all, we want the clinical men to become interested. I am sure that a very important step has been taken in forming this organization. I dare say that many of us will live to be proud that we were present at this first meeting of the Association."

Dr. C. E. deM. Sajous, of Philadelphia, called attention to the fact that relatively too much attention had been devoted to the pharmacology of the endocrine glands, which is only one phase of the subject. He especially emphasized the importance of the glands in pathology, and stated that many relationships on all sides are suggestive of the possibilities of endocrine pathology. He said this was emphasized in the nervous system, the hepatic functions, and in various other organs.

Dr. Hoxie, Chairman of the Organization Committee, presented a draft of the Constitution and By-Laws which was adopted. The election resulted in the choice of the following officers and members of the Council.

OFFICERS

President	C. E. deM. Sajous, of Philadelphia
Vice-President	Wm. Seaman Bainbridge, of New York
Secretary-Treasurer	Emil Goetsch, of Baltimore

COUNCIL MEMBERS

For one year	*For two years*
F. M. Pottenger, Los Angeles	L. R. DeBuys, New Orleans
L. F. Barker, Baltimore	Walter B. Cannon, Boston
Judson Daland, Philadelphia	G. H. Hoxie, Kansas City

For three years

John B. Potts, Omaha
Walter Timme, New York
R. G. Hoskins, Chicago

The first Council meeting was held on the evening of June 4, 1917, after the Annual Meeting of the Association. The following members of the Council were present: Doctors Barker, Hoxie, Pottenger, Potts, and Timme, and, *ex officio*, Sajous and Bainbridge.

The Council organized by electing Dr. Hoxie as Chairman, and Dr. Goetsch as Secretary.

Dr. Barker suggested that one of the important functions of the new organization was the publication of a journal. He said:

That the time has come for the establishment of a journal devoted especially to the literature of the internal secretions can hardly be questioned by any one familiar with this literature. Despite the preoccupation of the civilized world with war, there have appeared in the past eighteen months nearly two thousand articles directly concerned with the endocrine functions. These vary in quality all the way from the most superficial to the highly abstruse. All classes, however, demand consideration. The useless articles should be catalogued and their content briefly indicated in order to save the time of busy men who might otherwise be led to consult them. To offer disparaging comment is by no means a pleasant task, but the declared purpose of the Association can not otherwise be carried out. Another class of articles is highly technical in character, of immediate interest only to the specialist. The nature of these also should be briefly indicated. A large number of the articles published, however, are of sufficient interest to endocrinologists in general to demand abstracting in greater or less detail. Finally, a considerable number of the highly technical contributions which appear in the specialized journals not accessible to many readers contain findings that should at once be made generally available. Even as an abstract journal alone, there is abundant opportunity for useful service.

It is hoped that a sufficient number of competent abstractors may be enlisted to 'cover' completely the hundreds of medical and scientific periodicals (proceedings), and books which publish material of endocrine interest. The magnitude of this task many readers possibly do not appreciate. As an indication, however, of what a modern journal of this sort requires, it may be mentioned that the American Chemical Society is now spending some $40,000 a year on the publication of *Chemical Abstracts*.

A Board of Editors was selected, consisting of C. E. deM. Sajous, of Philadelphia, and L. F. Barker, of Baltimore, for one year, and A. F. Beifeld, of Chicago, and G. H. Hoxie, of Kansas City, for two years.

The Council instructed Dr. Goetsch to enter into negotiation with Dr. R. G. Hoskins, of Chicago, with reference to assuming the editorship of the journal, and with Dr. Henry R. Harrower, with reference to assuming the responsibilities of business management.

The first number of ENDOCRINOLOGY appeared as of January 1917, under the Editorship of Dr. Harrower. Dr. Hoskins assumed the Editorship beginning with the July–September issue of 1917.

The Council decided that the purpose of the Association could best be carried out by incorporating, and proceeded to carry out the plan accordingly

The Certificate of Incorporation of the Association for the Study of Internal Secretions was filed in the office of the Secretary of State of Delaware on the thirty-first day of January, 1918. The incorporators were: C. E. deM. Sajous, of Philadelphia; and L. F. Barker and Emil Goetsch, of Baltimore.

At a meeting held in Wilmington, Delaware, February 14, 1918, the incorporators confirmed as Officers and Directors those who had been chosen at the New York meeting.

The first Council Meeting after incorporation took place at the home of Dr. L. F. Barker, Saturday evening, May 23, 1918, in Baltimore, Maryland.

Dr. Hoxie had resigned as Chairman of the Council, and Dr. Walter Timme was elected to fill the vacancy. Dr. Goetsch was elected Secretary.

At this meeting the resignation of Dr. Harrower as Managing Editor was received and accepted, and Dr. F. M. Pottenger, of Monrovia, California, was designated as representative of the Association on the Pacific Coast. Mr. Paul Mason was elected Assistant to Dr. Pottenger in the publicity work of the Association in the West.

The Second Annual Meeting was held at the Blackstone Hotel in Chicago, June 10, 1918, at which time four papers were discussed as follows:

PRESIDENT'S ADDRESS: Comparative analysis of coordinated data in the study of internal secretions. *Chas. E. deM. Sajous, Philadelphia*
The thyroid hormone and its relation to the other ductless glands. *E. C. Kendall, Rochester*
A new pluriglandular syndrome. *Walter Timme, New York City*
The use of adrenal products in Addison's disease. *Judson Daland, Philadelphia*

The following officers were elected at this meeting:

President	Lewellys F. Barker, Baltimore, Maryland
First Vice-President	T. Brailsford Robertson, Berkeley, California
Second Vice-President	H. S. Plummer, Rochester, Minnesota
Secretary-Treasurer	F. M. Pottenger, Los Angeles, California

Members of the Council for three years:

C. E. deM. Sajous, Philadelphia
Judson Daland, Philadelphia
F. M. Pottenger, Los Angeles

At the Third Annual Meeting, held at Atlantic City, June 9, 1919, five papers were given as follows:

PRESIDENT'S ADDRESS: Remarks on the function of the suprarenal glands as revealed by clinical and pathological studies of human beings, and by experiments on animals. *Lewellys F. Barker, Baltimore*
The denervated heart as an indication of adrenal secretion in pain, asphyxia and excitement. *Walter B. Cannon, Boston*
Diabetes in children. *Isaac A. Abt, Chicago*
Functions of the thymus gland. *Edward Uhlenhuth, Rockefeller Institute, New York City*
Endocrine literature. *R. G. Hoskins, Chicago*

At this meeting all officers were re-elected with the exception of Dr. Robertson, First Vice-President. He was leaving for Australia, and Dr. Timme was elected in his stead.

At this meeting it was decided to change the articles of incorporation and the By-Laws to limit the powers more suitably to the needs of the Association. This was carried out during the year 1919.

These early programs show the paucity of subjects for discussion during these first meetings. It indicates how undeveloped the subject of endocrinology was at the time.

The Fourth Annual Meeting of the Association was held at the Hotel Gruenwald, New Orleans, on Monday, April 26, 1920. The increased interest in the Association was shown by the fact that three sessions were held at this meeting and more than five hundred people attended. Furthermore, this meeting marked a turning point in the influence of the Association. During the next two decades the most conservative workers in endocrinology were to affiliate themselves with the Association and vie in securing places on the annual programs. ENDOCRINOLOGY was chosen as the journal in which to publish the results of their researches.

But what of the future? What further has endocrinology to offer to medicine? This can be best forecast by looking back three-quarters of a century and seeing the status of medicine at that time and following the advances which have been made. By so doing we see that endocrinology is filling one of the blank spots of the past, and is explaining many problems in development and growth, many of the secrets

of metabolism, and at the same time is helping to solve some of the riddles in the realm of both emotional and physical reaction.

Scientific medicine in its early decades was dominated far more by the study of structure than function. The predominant effect of research in pathology and bacteriology was to emphasize disease and disease processes. When these subjects were at the height of their development clinical practice almost ceased to be a study of the patient. But there were men here and there who saw that no matter how great were the revelations made by the study of structure this would not solve the problems of medical practice. Such men were like voices crying in the wilderness, but they were scarcely heard.

After the beginning of the present century, however, the inadequacy of the anatomic approach began to be more fully appreciated, and at the present time it is quite fully realized that the chief interest in clinical medicine is the patient.

If the patient is to be our first interest, it is necessary to understand him as a complete functioning unit. We must seek to understand all mechanisms which are responsible for normal activity; for these are the same that show reaction in case of abnormal stimulation. Symptoms may be primarily due to disturbances in any single physiologic system—the endocrine, the nervous, or the electrolytic—but these are so intimately bound together that a disturbance in one is reflected in the others. The study of medicine then becomes a study of the disturbances in function which are produced by any and all morbific agents in any and all physiologic systems of the body. The proof of this fact is one of the contributions that the study of endocrinology is making to medicine, and the further elaboration of it is one of its greatest possibilities for the future.

The development of this physiologic phase of medicine is necessarily slower than that in which changes in structure could be followed by the microscope, because we are studying living, functioning processes in human beings who are subject to the effects of all the physical forces with which they come in contact, and all the emotional stresses which may affect them as individuals.

The early era in the development of physiology was confined largely to the study of the voluntary nervous system, and to the simpler disturbances in digestion, respiration, circulation, and the genito-urinary system. But these studies are relatively simple as compared with the complex physiologic problems which still remain to be investigated, such as those connected with the direct action and the correlating action of the products of the endocrine glands, the visceral nerves, the enzymes, and the electrolytes.

To make the subject more complex it must be borne in mind that higher mechanisms are made up of individual cells and as pathologic anatomy expresses itself in the cell so does pathologic physiology. How each cell can live and maintain its individuality in a complex organism and take from the humoral tissues which bathe it the substances which are suited to its existence and function and reject others is a matter of great importance and one of greatest wonder.

As endocrinology develops it reveals its intimate relationship to all physiologic systems of the body. It shows that our knowledge cannot be rounded out by developing from a standpoint of an individual system. While it may be necessary for us to conduct our research from the standpoint of endocrinology, the visceral nerves, the electrolytes or the enzymes, this will not satisfy the facts of physiology, for all physiologic systems dovetail intimately into each other. And while we are finding that the dominant activity of certain glands may seem to be expresssed in some special organ or some special function, there are wider activities which express them-

selves throughout the entire body that are of equal and in some instances probably of greater importance. Endocrinologic practice, therefore, does not belong to any particular specialty, but has a wide application to every phase of medicine.

Scientific medicine has awaited with impatience the development of endocrinology. It must await further the study of the visceral nerves, the enzymes, and the electrolytes and their integrating functions. Then and then only can we understand the individual from the time of conception through the various periods of growth, development, and activity through life. Endocrinology requires intensive laboratory research, but its final measure will be determined by its application to the benefit of the human race. A hormone may be ineffective by itself, but if study can reveal the entire physiologic mechanisms upon which it acts and upon which it depends for completion of its action, then it can be made of greatest importance to mankind.

HORMONES OF THE ADRENAL CORTEX

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THE ESSENTIAL CONTRIBUTION which subsequently led to the elucidation of the chemical nature of the hormones of the adrenal cortex was the use of organic solvents for the extraction of the active material from epinephrine and toxic substances which are associated with the hormones in the gland (1, 2). By this procedure extracts more or less potent have been made available since 1930, and it has now been shown that patients with Addison's disease and adrenalectomized animals can be protected by an extract of the adrenal cortex against the fatal consequences which are associated with a deficiency of this essential gland. Although this achievement, which indicates that the activity of the adrenal cortex can be substituted by the extract, is satisfactory and under certain conditions highly dramatic, there are many questions which arise in regard to the nature of the extract. During the past twelve years definite results have slowly accumulated which clearly show that the hormones of the adrenal cortex affect not only the distribution of inorganic ions and water but also the metabolism of fats, carbohydrates and proteins. Some aspects of the qualitative and quantitative determination of the physiologic activity of the various hormones (3) and some important factors which influence the potency of the extract are discussed.

Fractionation of Whole Extract

Through repeated distribution between water and benzene, the whole extract of the adrenal cortex is divided into two fractions. Some of the active compounds remain in the benzene; some pass completely into the water phase. If the benzene is removed and replaced with isopropyl alcohol, compound *A* separates within 24 to 48 hours. From the concentrated mother liquor of compound *A*, compound *B* will crystallize after the solution has stood for some days. In the mother liquor after the removal of compound *B* there are present a number of crystalline steroid derivatives, chief among which is compound *H*.

The compounds more soluble in water than in benzene are extracted from the aqueous solutions with chloroform, from which they can be crystallized. After compound *E* and related compounds have been separated there remains material easily soluble in the chloroform which has been described as the 'amorphous fraction' (4, 5).

From 1000 pounds of adrenal glands the following amounts of the various crystalline hormones and other steroid derivatives have been separated (table 1). These weights, however, cannot be regarded as indicative of the relative amounts of the various compounds in the gland itself. No attempt has yet been made to determine the quantitative distribution of all of the steroid derivatives.

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* President of the Association for the Study of Internal Secretions, 1930–1931.

Quantitative and Qualitative Differences of Hormones

Separation of the whole extract of the adrenal cortex into fractions and isolation of the hormones in crystalline form have permitted the demonstration that the various hormones have qualitative as well as quantitative differences in their effects. It therefore becomes essential to use a criterion for the determination of the physiologic activity which will be a measure of the specific effect produced by each hormone. If each of the fractions of the whole extract is assayed by the method in which adrenalectomized dogs are maintained in a normal condition and the minimal amount which is required is determined, then the 'amorphous fraction,' if properly prepared, can be

TABLE 1. DISTRIBUTION OF SOME OF THE STEROID DERIVATIVES OF THE ADRENAL CORTEX

Letter Designation		Derivative of Corticosterone	Milligrams of Compound in 1000 Pounds of Adrenal Glands
This laboratory	Reichstein's series		
A		11-dehydrocorticosterone	330
B	H	corticosterone	180
C	C	3,4,5-tetrahydro-17-hydroxycorticone	Less than 100
D	A	3,4,5,20-hexahydro-17-hydroxycorticosterone	Less than 100
E	Fa	11-dehydro-17-hydroxycorticosterone	340
F	M	17-hydroxycorticosterone	Less than 100
G	D	3,4,5-tetrahydro-11-dehydro-17-hydroxy-corticosterone	Less than 100
H	N	3,4,5-tetrahydro-11-dehydrocorticosterone	Less than 100
	L	3,4,5-tetrahydro-11,21-desoxy-17-hydroxy-corticosterone	Less than 100
	Q	desoxycorticosterone	Not present in significant amounts
	S	17-hydroxydesoxycorticosterone	Easily isolated but less than 100

shown to possess by far the greatest activity (6). This is because the 'amorphous fraction' affects primarily renal function. The daily requirement per kilogram of body weight to maintain the constituents of the blood within normal limits is less than 2 micrograms (7). On the other hand, if the influence on carbohydrate metabolism were used as a criterion, then the amorphous fraction would appear to be physiologically inert (8).

This wide discrepancy, which depends on the criterion used to determine physiologic activity, illustrates how divergent results can be obtained in various laboratories, and without citation of further details, it becomes evident that some methods which have been used in the past have been inadequate. The two qualitatively different effects, one on renal function, the other on carbohydrate metabolism, are independent and are distinctly separate one from the other. Each effect requires a special method of bioassay.

Perhaps the method which has led to the greatest confusion is the effect on growth of young animals (9). Young adrenalectomized animals fail to grow, and administration of extracts of the adrenal cortex will restore the rate of growth to normal. When, however, the influence on growth is used as a basis for a method of assay, insurmountable obstacles are encountered which make this method without value. For example, desoxycorticosterone will restore to normal the rate of growth of adrenalectomized young rats, and therefore presumably possesses physiologic activity characteristic of the adrenal cortex, but when desoxycorticosterone is tested in regard to its ability to affect carbohydrate metabolism (8, 10, 11) and to prevent certain forms of shock, it is found to be entirely lacking in activity (12).

In contrast to desoxycorticosterone, corticosterone and compound *E* do not have a favorable effect on the growth of young rats (13); given in large amounts they produce a loss in body weight. It would therefore be concluded that they do not possess significant activity characteristic of the adrenal cortex. Nevertheless, these two compounds produce the most striking effect on carbohydrate metabolism, on resistance to stress, toxic substances and shock (3). Many other examples of the inadequacy of methods for the bioassay of preparations of the adrenal cortex could be given.

Comparison of Potency of Extracts and Crystalline Hormones of Adrenal Cortex

Only those hormones which have an oxygen attached to carbon 11 produce an effect on carbohydrate metabolism, but all of the four hormones which have this structure have much less of an effect on renal function than do those steroid derivatives related to desoxycorticosterone.

In addition to these qualitative differences, significant quantitative relationships between the hormones in crystalline form and the hormones as they are present in the whole extract before fractionation have recently been shown. Quantitative determination of the amounts of compounds *A*, *B*, *E* and *F* indicates that not more than 0.05 mg. of compound *E* can be present in 1 cc. of extract which is made from 75 gm. of gland. Since the amount of each of the compounds *A*, *B* and *F* is of the same order, but not greater than the amount of *E*, the total weight of known hormones which affect carbohydrate metabolism cannot be greater than between 0.1 and 0.2 mg. per cubic centimeter of whole extract. The effect on carbohydrate metabolism of a unit volume of whole extract is many times the effect produced by the hormones which are contained in the unit volume when the hormones are given in crystalline form.

If the effect on carbohydrate metabolism of the whole extract is in fact produced only by compounds *A*, *B*, *E* and *F*, then the activity of these crystalline compounds must be greatly enhanced when they are present in the secretion of the gland. This suggestion was made many years ago, and at the time rested on the probability that a synergism exists between the hormones, so that in the secretion of the gland the effect is greater than that given by the hormones in pure crystalline form. It has been difficult to secure evidence which would establish the nature of the enhancement of the activity, but recently several new relationships have been established in my laboratory which greatly strengthen the hypothesis that the activity of the hormones is enhanced. Before these are described, another important factor should be discussed. This is the marked variability between various commercial preparations of extracts of the adrenal cortex.

One simple explanation for the high potency of some extracts when compared with others is the completeness with which the various hormones are extracted from the glandular material and are subsequently obtained in the aqueous extract. Although uniform amounts of *A*, *B*, *E* and *F* are not contained in all extracts, there seems little doubt that failure to extract the hormones is not the most important factor which determines the strength of the various preparations. The hormones exist in the gland in the aqueous phase. It is not necessary to extract the steroid derivatives from the fatty residues contained in the gland. Therefore, acetone, ethyl alcohol or methanol serves equally well for the precipitation of the protein and extraction of the hormones.

If the variability in potency of the extracts of the adrenal cortex does not depend principally on the completeness of extraction of the hormones, but is determined by other factors which modify the quality of the extract, it becomes a matter of first importance to examine whether the activity of the hormones is enhanced and if so to study the lability of the enhancement. This has been done as follows.

The high activity of a properly prepared solution is not modified if the solution is heated at 80° C. for 1 hour. The activity of the extract is not modified if an equal volume of benzene is added to the solution and the mixture is heated for 1 hour on the steam bath, so that the benzene actively boils and is returned by a reflux condenser. Vigorous aeration at room temperature for 30 minutes at a PH of 7.4 does not modify the activity. If an equal volume of benzene is added at PH 7.4 and the mixture is aerated for 30 minutes, the activity is not appreciably affected. These experiments show that the original high activity of the potent extract is not modified by heat and is not easily destroyed by mild oxidation or by hydrolysis.

A potent aqueous solution of the hormones of the adrenal cortex can be exhaustively extracted with benzene, the benzene can be removed in a vacuum, the residue dissolved in alcohol to which water is added, and the full original activity is found unaltered in the water solution after removal of the alcohol. However, if a similar benzene solution of the hormones is extracted with water, it can be shown that the water extract does not contain the full activity originally present. Examination of the benzene reveals the fact that the activity is not present in the benzene, and finally, a combination of the material extracted with water and that left in the benzene is little more active than the water extract itself. If the hormones are again taken into benzene and this is extracted with water and this procedure is repeated three or four times, as much as 75 to 80 per cent of the total activity may be destroyed.

The destruction of activity has been determined in two ways: *a*), through the effect on the renal function of adrenalectomized dogs and *b*), by means of a method recently devised by Dr. Reinecke (14) in my laboratory which is based on the deposition of glycogen in the livers of adrenalectomized rats. By use of these two methods it has been shown that the activity on carbohydrate metabolism and on renal function are both decreased, but that the activity on carbohydrate metabolism is more rapidly destroyed than is the effect on renal function.

The final proof that the high potency of the unfractionated extract is due to enhancement of the activity of the crystalline hormones would be secured if a method could be found which would restore the high potency to a solution whose activity has been decreased by repeated extraction of a benzene solution with water. To the present this has not been accomplished, and final conclusion on this important point must await further evidence.

It is possible that some steroid derivative, unisolated as yet, is responsible for the high activity on carbohydrate metabolism, but the probability that such a compound is present is greatly lessened by the demonstration that the original activity is stable to heat and to aeration at a PH of 7.4. It is not consistent, therefore, to assume that any compound is modified simply through distribution at room temperature between water and benzene.[1] From the aqueous phase after distribution between water and benzene it is possible to separate compounds *E* and *F* in good yield and from the benzene-soluble fraction, compounds *A* and *B* may be isolated in pure crystalline form. The stability of these crystalline compounds is such that it is impossible to explain loss of physiologic activity because of alteration in chemical structure. The accumulation of data of this kind diminishes the probability that any compound, as yet unknown, is responsible for the effect on carbohydrate metabolism. The new evidence makes it probable that the effect on carbohydrate metabolism is produced in the main by the four substances *A*, *B*, *E* and *F*, and that the high potency of the unfractionated extract is explained by the fact that these compounds exist in a form which has enhanced activity.

[1] The destruction of the high potency is not produced by benzene alone. Distribution between chloroform and water appears to be equally destructive.

A consideration of the nature of the amorphous fraction which affects renal function leads to a result which is much less definite. The activity is highly labile; it is almost completely destroyed by repeated distribution between water and benzene. However, the structure of the steroid which brings about the effect has not yet been shown. One definite statement can be made. The compound in the amorphous fraction which is so active on renal function is not desoxycorticosterone, since even in enormous doses this fraction does not cause retention of sodium and chloride and loss of potassium ion. It is also highly improbable that the steroid derivative in the amorphous fraction contains an atom of oxygen on carbon 11. Finally, whatever the structure of the steroid derivative may be, it exists in a form whose activity is greatly enhanced in the secretion of the gland.

Preparation of a Potent Extract from the Gland

This review is not suitable for a detailed description of a method for the preparation of a potent extract from the gland. There are, however, five topics which are of fundamental importance.

a). PH *and antioxidant effect.* Vitamin C and epinephrine, which occur in the adrenal gland, are both destroyed much more rapidly if the PH of the solution is increased in the absence of an antioxidant. The PH may be lowered and a suitable antioxidant introduced through the addition of sulfurous acid to the solution. At the time the frozen glands are ground and placed in alcohol, a solution of sulfur dioxide in alcohol is added so that the PH is reduced to about 4.5. The addition of sulfurous acid to the solution prevents the rapid oxidation of vitamin C and epinephrine, and it is noticeable that such solutions remain almost colorless for a much longer time than do untreated solutions.

b). *Temperature.* The hormones of the adrenal cortex are present in the water phase. Exhaustive extraction of the fatty material and phospholipids after removal of the water phase has shown that only insignificant amounts of any active steroid derivatives can be extracted from the fatty residues. If the glands are ground in the frozen state and are immediately placed in alcohol and the water-alcohol extract is pressed from the precipitated proteins, fat and phospholipids, at a temperature not greater than 5° C. practically all of the fats and lecithin remain in the solid phase. This is of great importance for the subsequent steps.

c). *Precipitation of lecithin.* Even though the extraction is made at a low temperature, a small amount of lecithin will dissolve, and experience has shown that when lecithin is precipitated during the concentration of the alcoholic extract, a certain amount of the steroid derivatives are carried out with the lecithin. The amount which is removed by precipitation of the lecithin can be reduced practically to zero if the lecithin is removed from solution while a high percentage of alcohol is still present. If solid sodium chloride is added to the alcoholic extract and the alcohol is concentrated in a vacuum at a temperature of the boiling solution not greater than 25° C. all but traces of lecithin are precipitated as soon as the concentration of alcohol reaches 45 per cent. If the concentration of the alcoholic solution in a vacuum is interrupted at this point and the solution is cooled to 5° C. and filtered, practically all the lecithin can be removed without loss of any of the steroid derivatives.

d). *Removal of last traces of lecithin.* If a water solution of the extractives of the adrenal cortex is treated with an organic solvent, such as benzene or chloroform, the amount of epinephrine which is dissolved in the benzene or chloroform is determined by the amount of lecithin present. If all of the lecithin is removed, then all traces of epinephrine can be washed out of the chloroform with dilute acid. Removal of the last traces of lecithin is easily accomplished by further concentration of the original

alcohol extract, after the first separation of the lecithin has been filtered out, so that not more than 25 per cent of alcohol is present. If the concentration of the extract is stopped, and the solution is allowed to stand at 5° C. overnight, a slight turbidity develops. The addition of barium chloride is satisfactory for the coagulation of this material and subsequent filtration yields a sparklingly clear, slightly yellow solution which is entirely free from lecithin. In addition, the barium chloride precipitates appreciable amounts of colored material; this improves the appearance of the final preparation. None of the steroid derivatives are carried out of solution by precipitation with barium chloride.

e). Removal of the hormones from water. The solution is extracted three times with one-sixth its volume of chloroform. The chloroform is then washed with water which contains sodium chloride. This removes any traces of epinephrine present in small droplets of the solution which are carried along mechanically in the chloroform. The hormones are from 30 to 100 times more soluble in chloroform than in water. Therefore, only traces of active material are removed when the chloroform solution is washed. The presence of salt decreases the solubility of the hormones in water, but as a final precaution the water is backwashed with chloroform.

One of the most important details is associated with removal of the chloroform. A large part of the enhanced activity will be destroyed unless the hormones are transferred from the chloroform to water in a manner which will preserve the high potency. The concentration of the chloroform is carried out immediately after the hormones have been extracted from the water. This is done in a vacuum without the addition of water to the flask which contains the chloroform. After most of the chloroform has been removed, alcohol is added to the chloroform which remains. The last of the chloroform is then distilled off, and finally, water is added to the alcohol. The alcohol is in turn completely removed. In this way the hormones are always in solution, first in chloroform, then in alcohol and finally in water, and at no time have the hormones been distributed between two phases. The final water solution is filtered from a small amount of insoluble material. Such a preparation is colorless, practically free from odor and possesses a high potency.

Influence of Whole Extract and Compound E on Carbohydrate Metabolism

In a study of the relationship between the pituitary, the pancreas and the adrenal glands Houssay and Biasotti (15), Long and Lukens (16) and others have shown that the suppression or ablation of the adrenal cortex exerts an ameliorating effect on the severity of the condition which is produced by a deficiency of insulin. Early attempts to increase the severity of the diabetic state through the use of extracts of the adrenal cortex failed. It now seems most probable that the reason for the failure was the low potency of the extracts available at that time.

In 1937, through the use of crystalline compounds *A*, *B* and *E*, Long, Fry and Thompson (17) were able to demonstrate a great increase in the severity of the diabetes in partially depancreatized rats. In such rats sufficient pancreatic tissue was present to prevent, in most cases, the excretion of sugar in the urine, even though the rats were given a liberal diet. The addition of compounds *A*, *B* and *E* brought about glycosuria and also an increase in the excretion of nitrogen. This indicated that the excretion of sugar was associated with a breakdown of proteins (11).

Wells, in my laboratory, subsequently demonstrated a striking effect of compounds *A*, *B* and *E* on the glycosuria produced by phlorhizin in adrenalectomized rats. The increase in the excretion of glucose was associated with an increase in the excretion of nitrogen, and the ratio of glucose to nitrogen 3.6 indicated the usual re-

sponse to phlorhizin. In the absence of crystalline hormones *A*, *B* and *E* the rats could not survive the administration of phlorhizin, and the amount of glucose and nitrogen excreted was not more than one-third that excreted after the administration of the hormones (18). In all these experiments the apparent source of the extra sugar was protein, since the ratio of glucose to nitrogen was 3.6.

In addition to the demonstration that the hormones of the adrenal cortex induced gluconeogenesis, Long and his co-workers also showed indirectly that these hormones interfered with the utilization of glucose (11). Thorn and his associates (10) observed a decrease in the respiratory quotient which was produced by potent extracts and crystalline hormones of the adrenal cortex, and also an apparent increase in the rate of utilization of glucose in untreated patients with Addison's disease and in adrenalectomized animals. They suggested that the hormones of the adrenal cortex interfered with the utilization of glucose. Greaves (19) has shown that compound *E* decreases the respiratory quotient in rats.

Ingle (20) and Ingle and Thorn (21) have shown that although compound *E* administered to normal rats on a high carbohydrate diet or to partially depancreatized rats produced glycosuria and an increase in the excretion of nitrogen, not all of the glucose could be accounted for on the basis of the formation of glucose from protein. The assumption was made that compound *E* inhibits the utilization of carbohydrate or that it alters the interconversion of foodstuffs in addition to its effect on the catabolism of protein.

Closely related experiments were carried out by Wells and showed that one possible explanation for the influence on carbohydrate metabolism induced by compounds *A*, *B* and *E* is the antagonistic action of these hormones to insulin (18) and Grattan and Jensen (22) independently demonstrated that the convulsions produced by insulin could be counteracted by the crystalline hormones of the adrenal cortex. Throughout these experiments the evidence in regard to the decreased rate of utilization of carbohydrate has been indirect. Recently some results have been obtained with an adrenalectomized, depancreatized dog which provide direct evidence for this effect. For the necessary surgical operations I am greatly indebted to Dr. F. C. Mann.

The adrenalectomized, depancreatized dog was maintained by the daily administration of 12 units of insulin, all of which was given at one time. No protamine-zinc insulin was used. Nevertheless, in the absence of the adrenal cortex, the single injection of 12 U of insulin produced an effect throughout 24 hours and the blood sugar on the next morning had not reached the level seen in untreated diabetes. The dog was also fed once a day at the time of administration of insulin, and was maintained with a small amount of extract of the adrenal cortex. On such a regimen only traces of sugar would appear in the urine if as much as 14 U of insulin were given. With 12 U of insulin the glucose in the urine varied between 5 and 10 gm. If compound *E* (10 mg.) was administered daily to the dog, there was a prompt increase in the excretion of glucose up to 51 gm. of glucose in 24 hours on the third day of administration. The high level of glycosuria was maintained as long as compound *E* was given, but when the administration was stopped the glycosuria returned to the level of 5 to 10 gm. a day.

Associated with the increased glycosuria there was a rise in the level of the blood sugar to the diabetic level, 350 mg. per 100 cc. When the administration of compound *E* was stopped, the concentration of glucose in the blood returned to between 200 and 250 mg. per 100 cc. Samples of blood were always taken 24 hours after the single injection of 12 U of insulin.

These results indicate the striking effect of compound *E* on carbohydrate me-

tabolism, but perhaps the most significant observation is that during the period when the glucose was increased from 10 to 50 gm. per day, almost no extra nitrogen was excreted in the urine. The source of the extra sugar therefore was not endogenous protein. These experiments afford direct evidence that in the presence of minimal amounts of insulin, compound *E* can suppress the utilization of glucose. This is associated with a rise in the level of the blood sugar and by the excretion of glucose. The source of the glucose was apparently the food and there was no evidence that compound *E* under these conditions induced gluconeogenesis.

The administration of from 2 to 3 mg. of compound *E* a day to a partially depancreatized rat may cause an increased excretion of both glucose and nitrogen in the urine. But this is an extreme condition, and the gluconeogenesis is doubtless brought about by the relatively enormous doses of compound *E*. The results of the depancreatized, adrenalectomized dog, however, show that with smaller amounts of compound *E* per kilogram of body weight, definite inhibition of the utilization of glucose is produced and the dosage used permits a clear demonstration of this effect without gluconeogenesis.

The administration of small amounts of insulin completely blocked this influence of compound *E*. One interpretation of the result is that compound *E* exerted its effect primarily through its antagonism to insulin and thereby suppressed the utilization of glucose. Although there was a rise in the concentration of glucose in the blood and excretion of glucose in the urine, compound *E*, under the conditions of the experiment, did not stimulate the overproduction of glucose from protein.

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FUNCTIONS OF THE ADRENAL CORTEX

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Evidence is accumulating that the adrenal cortex produces several hormones by means of which it carries on its various functions. The crystalline compounds that have been isolated probably are more stable derivatives of an unknown number of original hormones.

Unfractionated extract contains the hormones in as nearly a natural state as it is possible to obtain them. Through a study of this extract and its fractions we are beginning to understand the functions of the cortex. Some functions involve the organism as a whole while others have a more specific relation. The former include regulation of permeability and electrolyte balance as well as control of growth and energy metabolism. The latter include detoxification, control of pigmentation, specific effect on the nervous system, influence on milk production, and the effects on vitamin deficiencies.

Regulation of Permeability

A disturbance in permeability was noticed early in the study of adrenal function (1). The loss of blood plasma after adrenalectomy is due to increased permeability of the capillaries (2). Associated with it is a redistribution of water throughout the body. The cells absorb water—tissue cells from the interstitial fluid (3), and erythrocytes from the plasma (4). Winter and Hartman (5) found that skeletal muscle from adrenalectomized rats shifted its water more readily in either direction than muscle from normal animals. Water entered more rapidly from hypotonic solution and left more rapidly in hypertonic solution. The negative results of Ponder and Gaunt (6) might be explained by the difference in the stage at which their animals were tested. In a late stage the limit of imbibition may be approached. The Angerers (7) found that adrenalectomized frogs absorbed an abnormal amount of water. Some of this excess went to skeletal muscle so that it was able to imbibe less water from hypotonic solution than did normal muscle. Angerer (8) found that adrenalectomy increased the rate of permeability of frog's skin to radioactive potassium. This change appeared before signs of adrenal insufficiency were manifest.

Swingle and his associates have done much to elucidate the role of the cortex in the control of the circulation. They (9) showed that the intravenous injection of either whole blood or serum failed to improve the condition of an adrenal-insufficient animal. The transfused fluid soon leaked into the tissues giving rise to edema. Injection of adequate adrenal extract prevented this escape or produced a redistribution of the water if it had occurred (10). Indeed, Menkin (11) has found that adrenal extract inhibits the increased permeability produced in normal animals by leukotoxin.

Complete adrenal insufficiency leads to a slow, progressive decline in blood pressure, eventually to the point of collapse. The heart may become so feeble that there

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is a marked drop in cardiac output (12). The degree of vasomotor response is reduced and certain blood vessels may become intensely dilated (13). These changes, together with the loss in plasma volume, are responsible for circulatory failure. Although adrenal extract will sometimes restore plasma volume and blood pressure without extra fluid (14), the administration of fluid may be required for complete restoration.

Regulation of Electrolyte Balance

The evidence is very good that in unfractionated extract sodium retention is due to a distinct hormone. This hormone can be rendered ineffective by the development of refractoriness through repeated injections (15, 16). Such an extract fails to cause sodium retention, yet appears to retain its other properties. Adrenalectomized animals, adequately treated with an extract to which they have become refractory, excrete sodium so that its concentration in the plasma reaches the level characteristic of adrenal insufficiency. There is no other demonstrable deviation from the normal. The blood pressure does not fall, nor is there any change in permeability as indicated by a normal plasma volume (17). It is evident, therefore, that there are at least two factors in the extract, one responsible for sodium retention and the other which can maintain permeability relationships. The second factor was tentatively called cortin.[1] However, this does not mean that the sodium factor is unimportant, for without it much larger amounts of the other fraction are required. Moreover, sodium factor or sodium-retaining substances alone can maintain adrenalectomized animals as well as patients with Addison's disease under basal conditions for a considerable period.

Sodium factor seems to be more important in some species than in others. In the marmot and opossum, no loss in sodium occurs after adrenalectomy (19). In the elasmobranch, there is an increase in plasma sodium after interrenalectomy (20). We have been unable to obtain any effect of desoxycorticosterone on the electrolytes in the latter group of fishes.

There is additional evidence of the importance of the adrenal in relation to sodium. Normal animals respond to a low sodium diet first by an increase and then a decrease in size of the gland (21). Also, if adrenalectomized animals are to be maintained on a salt-free diet, they require massive doses of extract (22).

So far it has been impossible to isolate the sodium factor by precipitation without modification of its properties. The simplest technique employed abolishes the power to produce refractoriness although the capacity for sodium retention is retained (23).

Disturbance in sodium balance is often accompanied by an increase in potassium in the plasma. However, reduction in plasma sodium can occur without change in plasma potassium. Indeed, in the presence of an adequate cortin fraction without sodium factor, the plasma sodium may fall to the level characteristic of complete adrenal insufficiency without change in potassium. The rise in plasma potassium does not seem to be due to lack of a specific factor since with sodium factor alone there may be very little change in plasma potassium (17).

Excessive loss of sodium through the kidney accounts for low plasma sodium but renal retention of potassium only partially accounts for the rise in potassium. Loeb, Atchley, Benedict, and Leland (24) found that the concentration of potassium in the plasma bore no relation to the potassium balance. The release of potassium by the cells is possibly a direct result of the absence of adrenal secretion, for Tipton (25) found that adrenal extract decreased the loss of potassium which occurs upon stimulation of normal muscle. Perhaps tissues like the liver (26), which most readily yield

[1] The term cortin was originally used (18) to designate the essential hormone of the adrenal cortex, not the extract itself as some have interpreted.

potassium, furnish the material for plasma increase. Prolonged asthenia itself may lead to loss of potassium from the muscle cells since it has been shown that this occurs in amyotrophic conditions (27). Accompanying the plasma increase, there is a rise in potassium in skeletal muscle (28, 29) and in the erythrocytes (4). This may be secondary to the plasma increase.

Calcium metabolism may become upset in adrenal insufficiency. Rogoff and Stewart (30) found an increase in serum calcium while Schour and Rogoff (31) observed characteristic changes in the predentin of rats' teeth after adrenalectomy.

Relation to Sulfur Metabolism

A number of observations point to participation of the adrenal in sulfur metabolism. The possible significance of this cannot be ignored.

A disturbance in endogenous sulfur metabolism is indicated after adrenalectomy by an increase in the sulfur in the blood (32) and skin (33), as well as in the sulfur excreted by the kidney (34). Adrenal extract is said to reduce the blood sulfur in both adrenalectomized (35) and normal (36) animals.

The adrenal appears to take sulfur from the blood. Loeper, Garcin, and Lesure (37) found more sulfur in the blood entering the gland than in that leaving it while Lurie (38) not only found relatively more sulfur in the adrenal than in any other organ but observed that the adrenal took up more sulfur after sulfur inunction.

An organic sulfur compound which may have its origin in the adrenal is glutathione, since perfusion with glutamic acid and cystine increased the glutathione content of the gland as well as the perfusion fluid (39). Moreover, the adrenal is rich in glutathione (40) and contains proportionally more reduced glutathione than any other organ (41). Unilateral adrenalectomy is said to increase the glutathione content of the remaining adrenal as much as three-fold (41). Complete adrenalectomy decreased blood glutathione (42) while injection of adrenal extract increased it (41).

Control of Energy Metabolism

Following the removal of both adrenals, basal metabolism falls about 25 per cent (43, 44). Any treatment which will maintain an animal in good condition will also maintain the basal metabolism at the normal level. Thus, under certain conditions, the basal metabolic rate can be maintained within normal limits by various adrenal fractions as well as by sodium salts (45).

The metabolic disturbances present in adrenal insufficiency might be due to changes in the circulatory or nervous systems. However, a study of isolated tissue shows that these factors alone are not responsible. Estrada (46) found that the oxidation processes in muscle were nearly always lowered by adrenalectomy. The metabolism of kidney (47) and liver (48) slices was lowered as much as 25 per cent. Himwich et al. (49) found that the respiratory quotient of the brain was decreased even in the presence of glucose.

Reduction in enzyme activity may be a factor in the disturbed metabolism. The deamination of amino acids by kidney tissue is reduced. (50) This can be returned to normal by adrenal extract or by desoxycorticosterone (51).

The rate of oxidation of pyruvate, succinate, and citrate by the kidney (51) and by the liver (48) is decreased after adrenalectomy.

Carbohydrate Metabolism

It has been shown that adrenalectomized animals maintained in good basal condition by sodium salts or by sodium retaining substances do not meet the extra de-

mands made by cold or by exercise. This is due in part to the failure of glyconeogenesis because glucose infusions help to restore the work capacity of adrenalectomized rats (52). Cortical extract not only restores the power of glyconeogenesis, but, if given in large amounts, may increase liver glycogen several times that found in normal animals (53). Corticosterone and related compounds with an oxygen in the C_{11} position also possess this property (54, 55).

All processes involved in carbohydrate metabolism appear to be retarded in adrenal insufficiency. The speed of absorption is reduced (56). It is well-known that blood sugar may fall (57). Moreover, it has been found that symptoms of hypoglycemia appear at higher blood-sugar levels in patients with Addison's disease than in normal individuals (58). Production (59) and removal (60) of lactic acid may be delayed so that the amount of this substance in the blood may be increased or decreased, depending upon which effect predominates.

The utilization of carbohydrate may be increased in adrenal insufficiency. It was found that the defective glyconeogenesis could account for not more than half of the difference in carbohydrate disappearance between normal and adrenalectomized animals during fasting. Moreover, the adrenalectomized animals failed to deposit glycogen after glucose injection. The glucose tolerance curves, however, were normal (61). Therefore, more glucose must have been oxidized. Conversely, it has been shown (62, 63) that adrenal extract depresses the oxidation of carbohydrate.

Adrenalectomized dogs kept in good condition and showing normal basal metabolism responded by a marked delay in the rate of development of the specific dynamic action when fed a large amount of dextrose. This was explained in part by a slower rate of absorption as indicated by sugar tolerance curves determined after oral and intravenous administration (45). Another factor may be reduction in the rate of conversion of dextrose to glycogen (64, 65). It is obvious that treatment sufficient to maintain basal condition is inadequate for full functional activity.

Protein Metabolism

Little is known concerning the relation of the adrenal to protein metabolism. The high blood-urea of adrenal insufficiency seems to be explained, at least in part, by the impaired excretory ability of the kidney. The reduced rate of growth in young animals after adrenalectomy may be due to retarded anabolism of protein as well as other factors. The increased nitrogen excretion accompanying the injection of adrenal extract in both normal and adrenalectomized fasted animals parallels the rise in liver glycogen. The amounts suggest the conversion of protein to glucose (62). The power to deaminize amino-acids is reduced after adrenalectomy (50). The specific dynamic action of protein is lowered (66) and retarded even in adrenalectomized dogs kept in good condition by adrenal extract, as was found for carbohydrate (45).

Lipid Metabolism

The rôle of the adrenal cortex in lipid metabolism is not very well understood. According to some authors, adrenalectomy reduces the rate of lipid absorption (67). In rats maintained on salt solution, this is said not to occur (68, 69). Verzar's hypothesis that the reduced absorption is due to failure of phosphorylation has not been supported by the work of other investigators (70). Adrenalectomized rats are not fattened by fatty foods (71) according to Verzar and Jeker (72) because of the failure of neutral fat synthesis from the fatty acids absorbed by the intestine. Moreover, there seems to be an inability to mobilize liver fat in adrenal insufficiency since Zummo and Infantellina (73) found that the phospholipid fatty acids and phosphorus

of the liver did not change in adrenalectomized rats exposed to cold as they do in normal animals.

Further evidence that something is missing after adrenalectomy that usually influences fat metabolism is shown in the failure of fatty infiltration of the liver following pancreatectomy (74), partial hepatectomy (75), and phosphorus poisoning (76). Adrenalectomy has also been shown to reduce the amount of liver fat during fasting following a high fat diet (77).

There is a marked delay in the rate of development of the specific dynamic action of fat in adrenalectomized dogs maintained in good condition by treatment with various adrenal preparations. In other words, they are not completely normal although their basal metabolism may be normal (45).

MacKay and Barnes (78) believed that the ketolytic activity of the organism was increased after adrenalectomy because the fasting ketosis of the pregnant animal was abolished. Moreover, ketonuria, after feeding salts of diacetic or β-oxybutyric acid to fasting rats, was reduced by adrenalectomy (79).

Findings concerning the relation of the adrenal to cholesterol are so contradictory that future developments must be awaited.

Control of Growth

Adrenalectomy retards growth. This is shown especially in young animals. It has also been observed in the slow healing of fractures (80) or of other wounds. These effects can be accounted for in part by the general disturbances of metabolism. This question deserves further investigation.

Relation to Detoxification

The lowered resistance to toxins during adrenal insufficiency might be a result of the lowered metabolic activity of the organism. The whole protective mechanism appears to be less responsive after adrenalectomy. There is a striking depression in antibody formation (81). The quantity of antigen necessary to yield maximum antibody titer becomes many times that required in normal animals (82). Moreover, small doses of antigen, harmless to normal animals, may be fatal (83).

The well-known increase in susceptibility to anaphylactic shock after adrenalectomy (84) may be due in part to a cortical factor. Adequate doses of adrenal extract are said to increase the protection in normal animals (85, 86). This may be accomplished by increasing the resistance to histamine (87).

In view of the foregoing observations, the marked reaction of the adrenal cortex, as shown histologically, to bacterial and other toxins may indicate an attempt to meet a demand for an increased output of hormones.

Control of Pigmentation

One of the cardinal signs of Addison's disease is the pigmentation which is usually present in greater or lesser degree. Pigmentation is intensified in regions stimulated by light or pressure. The removal of pigment appears especially to be at fault because a summer suntan which fails to disappear during the winter may be the first sign of the disease. It might be due to disturbance in enzyme action. It is unaccounted for by the absence of any known adrenal factor and probably specific since it may occur many years before the onset of serious symptoms. It seems to be peculiar to man because of the thousands of adrenalectomized animals observed, no positive instances of pigmentation have developed. Unfractionated extract contains a factor which can cause the disappearance of the pigmentation in some cases.

Relation to Vitamin Deficiencies

The adjustments which occur in inanition or in certain vitamin deficiencies seem to involve the adrenals. They usually increase in size during inanition (87) and in vitamins A (89), B_1 (90), C (91), and D (92) deficiencies. Cortical extract free from the respective vitamin delays the onset of scurvy and of avitaminosis B_1. It does so while some vitamin is available in the organism but is ineffective when the vitamin has completely disappeared (90, 93). This might be due to protection of the vitamin against destruction.

Relation to Lactation

Cortilactin, a factor described first by Brownell and her associates (94) has recently been prepared by isoelectric precipitation. It enables a mother rat kept in good condition by other adrenal fractions to lactate adequately so that all of her pups survive with a normal growth curve. Cortilactin does not affect glyconeogenesis (95). Dr. Robert Gaunt (personal communication) has obtained full replacement by means of Kendall's compound *E*. Therefore, it seems quite possible that more than one factor may be effective in lactation.

Influence on the Nervous System

Elliott (96) suggested long ago that the symptoms of adrenal insufficiency were due to reduced activity of tissues especially innervated by the sympathetic nervous system. Hoskins (97) also found that adrenal insufficiency resulted in depression of irritability in the sympathetic nervous system. Nowak (98) found that the carotid sinus reflex was practically abolished. Armstrong et al. (99) showed that splanchnic nerve stimulation had negligible pressor effects in the terminal stage of adrenal insufficiency. These might be secondary effects, although Burns, Reese and Sellman (100) detected degenerative changes in the sympathetic ganglia in this condition.

Clinically there is evidence that the nervous system may be directly affected by the adrenal. Although the psychoneural disturbance in Addison's disease is usually accompanied by general asthenia it may precede other signs and symptoms. It consists of mental fatigue, insomnia, restlessness and abnormal reactions in the sensory apparatus. This change in the mental aspect may so overshadow the rest of the syndrome that patients with Addison's disease have been treated by the psychiatrist for their mental condition without recognition of the disease.

The effects of cortical extract on certain types of patients is also suggestive. These were organic neurological cases. There was not only improvement in the motor functions, but also a rather striking change in the mental status. Cheerfulness, a sense of well-being, and even euphoria replaced depression. Hyperirritability disappeared along with improvement in the gastrointestinal functions and sleep. In one case the improvement persisted for more than sixteen days after cessation of treatment (101).

Objective evidence that cortical preparations can influence the nervous system was obtained by Liddell and his associates (102). A neurosis was produced by a conditioned motor reflex in sheep. This was characterized by extreme excitement, uncooperative behavior and spontaneous, nervous twitching movements. The extract, after a few injections, quieted the animal, increased the vigor of the conditioned reaction and decreased the frequency of the nervous movements. The effects persisted for more than twenty-four days after the last injection. A similar persistence of a few weeks after extract had been discontinued has been noted in the reduction of oxygen consumption produced by adrenal extract (103, 104). This might also act through the nervous system. Hoskins and Freeman (105) likewise observed persistence in the

blood pressure rise for a few days after administration of adrenal cortex had ceased. Their subjects were schizophrenic patients. The cardiovascular reaction to environment and to exercise was increased during treatment.

The beneficial effect of adrenal extract in fever therapy may be due to action on the nervous system. Edelmann and his associates (106) have found that adrenal extract alleviates many of the ill effects of fever therapy such as weakness, nausea, and vomiting. Recovery was more rapid and repeated treatments were better tolerated. Extract had no appreciable effect on the plasma volume as indicated by hematocrit values. While there was possibly a reduction in electrolyte excretion in the perspiration, it was insufficient to account for the results.

A survey of the disturbances in adrenal insufficiency leads to certain generalizations.

The changes which occur after adrenalectomy are essentially of a metabolic nature. This is confirmed by postmortem findings. We have performed autopsies on scores of adrenalectomized animals allowed to succumb from gradual reduction of treatment. As in Addison's disease, no organic lesion was found which could explain the fatal outcome. Talbott et al. (107) have recently found no consistent structural changes in the kidneys of patients who had died of adrenal insufficiency although the renal function had been significantly impaired.

These changes are quantitative rather than qualitative in character, normal processes being merely retarded. The consequences may become serious only when continued for a long time or when stress creates an unusual demand.

Of the many functions involved in adrenal insufficiency it is difficult, at the present time, to fix upon any one as the primary cause of the condition because of the close interrelationships which exist.

We find an indication of the production of a number of hormones by the adrenal cortex in the variation of the picture presented by different individual cases of Addison's disease. In some there may be lowered blood pressure and other signs without change in electrolyte balance. In another group the electrolyte balance may be the predominant modification while, occasionally, central nervous disturbances or faulty pigment metabolism stand out. In complete adrenal insufficiency, however, as after adrenalectomy, this distinction does not occur.

Many disturbances contribute to the asthenia. Control of permeability, influence on enzyme action and regulation of electrolyte balance are certainly general cellular functions and are probably responsible for some of the disturbances in energy metabolism. They can account, at least in considerable measure, for the asthenia of the muscular and nervous systems, but the possibility of direct effect through the central nervous system must also be considered.

Control of Adrenal Cortical Activity

Because of the atrophy resulting from hypophysectomy and because of the effect of the injection of adrenotropic preparations from the pituitary, it has been concluded that the pituitary gland is the 'master' of the adrenal cortex. However, the adrenal cortex can be influenced by other factors. Miller and Riddle (108) showed that insulin produced enlargement, mitosis, and cellular activity in the adrenals of pigeons without their pituitary glands. The cytological changes in the cortex were identical with those induced by adrenotropic pituitary extracts. The adrenal cortex also functions independently. After hypophysectomy, there is still enough activity to prevent death from adrenal insufficiency.

Thus, we see that the adrenal cortex is an independent organ which through its

influence on permeability, electrolyte balance, energy metabolism, enzyme action and nervous function plays an indispensable rôle in practically all activities of the organism.

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A DISCUSSION OF THE MECHANISM OF ACTION OF ADRENAL CORTICAL HORMONES ON CARBOHYDRATE AND PROTEIN METABOLISM[1,2]

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THE INTENSIVE WORK of the last few years has resulted in the accumulation of a large body of factual knowledge concerning not only the active principles of the adrenal cortex but also the effects produced by them, both in adrenalectomized and normal animals, on various phases of metabolism. These, coupled with the numerous observations on untreated adrenalectomized animals, would, it might be supposed, have served as a basis for a rather complete understanding of the mechanism of action of this endocrine gland. This, as all students of the subject are aware, is far from the case. Many features of the behavior of adrenalectomized animals as well as the effect of adrenal hormones in normal animals would appear to necessitate the acceptance of the view that the adrenal gland participates in the control of the inorganic and organic metabolism by the operation of two different types of hormones.

While this paper is largely confined to a discussion of the action of the cortical hormones on the organic metabolism, an attempt will also be made to point out that certain of these changes of necessity involve the inorganic metabolism, as well as to suggest that it should not be too hastily concluded that there is a hard and fast line of demarcation in the apparent twofold quality of the function of the adrenal cortex.

Before discussing the major changes observed in adrenalectomized animals or in normal animals given an excess of cortical hormone it is useful to outline the various types of active substances that have been isolated from the gland.

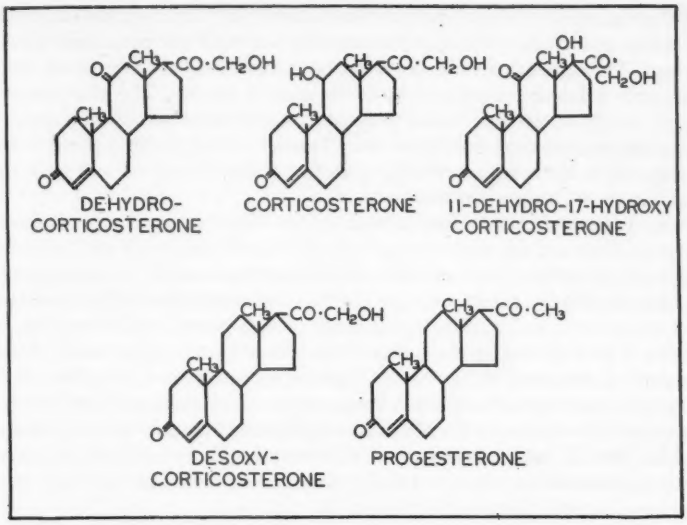
Active Principles Isolated From the Adrenal Cortex

a). The members of the corticosterone series are characterized by the presence of an hydroxyl or carbonyl group at carbon eleven. The three members of this group whose physiological properties have been chiefly studied are corticosterone, 11-dehydrocorticosterone and 11-dehydro 17-hydroxycorticosterone. These three substances will hereafter be referred to as the corticosterones.

b). 11-Desoxycorticosterone whose structure is very similar to that of progesterone is the only member of the adrenal steroids that has been synthesized. Although it is not the most abundant steroid in cortical extracts, its ready availability and widespread use appears to have led to an overemphasis of its importance in the normal mechanism of action of the adrenal cortex. Progesterone has also been isolated from cortical extracts.

[1] The experiments reported from the Department of Physiological Chemistry, Yale University School of Medicine, were supported by a grant from the Committee on Research in Endocrinology, National Research Council, and the Fluid Research Fund, Yale University School of Medicine.

[2] We are indebted to Dr. E. C. Kendall, of the Mayo Clinic, Rochester, Minnesota, for a generous supply of 11-dehydro 17-hydroxycorticosterone.



c). After removal of all of the crystalline steroids there still is present in the mother liquor certain unidentified compounds that possess biological activity. This fraction is termed by Kendall (1) "the amorphous fraction."

d). Besides progesterone, other steroids normally associated with the gonads have been identified. Among these are estrone and certain androgenic substances.

Influence of the Adrenal Cortex on the Organic and Inorganic Metabolism

The major alterations that are observed in adrenalectomized animals, or animals either normal or adrenalectomized that are receiving an excess of an active cortical principle, may for convenience be classified as follows.

Life maintenance. For an understanding of the mechanism of action of the adrenal cortex the necessity of this gland for the maintenance of life is probably the least informative. Adrenalectomized animals may die from a profound disturbance in their electrolyte and water balance, from hypoglycemia, or from an inability to resist the stresses associated with their normal habits of life. In the past too much attention has been paid to the immediate cause of death of these animals and too little to the changes that are the basis of its occurrence.

Water and electrolyte metabolism. Inabilities of the kidney to retain sodium chloride and to remove potassium from the body are now recognized as characteristic features of cortical insufficiency. The magnitude of these changes varies in different species being particularly evident in man and the dog, less so in the rat. In some species as the opossum and marmot they apparently do not occur since it is reported that the serum levels of sodium and chloride actually increase after adrenalectomy (2).

These alterations in the electrolyte regulation of the kidney are reflected by corresponding changes in the extracellular fluid, and a lowered sodium and an increased potassium content of the serum are found in those animals in which the electrolyte loss is most severe.

The administration of cortical extract or of certain adrenal steroids not only cor-

rects this situation in adrenalectomized animals but if given in excess to normal animals results in sodium, chloride and water retention while the potassium excretion is increased. This statement must, however, be expanded. In the first place, there is a quantitative difference in the activity of the adrenal steroids. The most active in its influence on the electrolyte balance is desoxycorticosterone followed by the steroids of the corticosterone type. It is reported by Kendall (1) that the amorphous fraction is also very active. Even more surprising is the fact that progesterone and the estrogens also induce these changes in normal animals (3).

Now it is evident that failure to maintain the electrolyte and water balance will soon cause death and also that any agent that alleviates the extent of this defect will be reflected by an increased survival. Consequently, it might be anticipated that the administration of a substance, even if not a specific principle of the adrenal cortex, which can return the electrolyte equilibrium towards normal would be of assistance. That this is so is shown by the fact that the administration of an excess of sodium, particularly if combined with a reduced potassium intake, to adrenalectomized animals, greatly prolongs their existence. Progesterone which induces electrolyte and water retention also maintains the life of adrenalectomized animals as does desoxycorticosterone. Both of these substances are, however, singularly ineffective in correcting the other abnormalities associated with adrenal insufficiency nor do they influence these phases of metabolism in normal animals.

The behavior of the estrogens is very interesting. Both the natural hormones and the synthetic substance diethylstilbestrol are active in normal animals, bringing about alterations both in the inorganic and organic metabolism. They are, however, of no value for the maintenance of life in adrenalectomized animals and there is reason to believe that their effects on normal animals depend on the presence of the adrenal cortex and the anterior pituitary.

Capillary permeability and the control of vascular tone. Swingle and his associates have published numerous papers which stress the view that all the electrolyte and water shifts associated with cortical insufficiency are not to be explained by progressive renal failure. They point out that the extreme susceptibility of adrenalectomized dogs to hemorrhage, fluid depletion by intraperitoneal injection of glucose, or the inability to dispose of excessive quantities of ingested water, reflect an incapacity of the animals to transfer fluid across the capillary walls. Consequently, the maintenance of the plasma volume by shifts of water (and salts) into and from the extra-cellular fluid which occurs so rapidly in normal animals is so reduced in its extent by cortical insufficiency that these animals soon succumb to conditions in which such adjustments are imperative. Only a few reports have appeared in which direct observations on the influence of adrenal steroids on capillary permeability have been made. Menkin (4) has reported that the permeability of capillaries damaged by the injection of irritants was altered by the local injection of desoxycorticosterone and cortical extracts. The alteration in capillary permeability was judged by the rate of diffusion of trypan blue from the site of irritation. Recently Fine and Fischman (5) have confirmed the decrease in the rate that trypan blue leaves the capillaries of the rabbit's ear after the injection of desoxycorticosterone but are not agreed that this represents an altered capillary permeability to water and electrolytes and quote several other types of experiments that they believe support their contention.

Freed and Lindner (6) repeated Menkin's experiments but found that while cortical extracts and corticosterone were effective in preventing the spread of the wheal, desoxycorticosterone was inactive. They conclude that the ability of adrenal steroids to maintain life is not dependent on their action on capillary permeability.

In recent years Swingle has extended his work to the study of the resistance of adrenalectomized animals to trauma of various kinds. He and his colleagues have shown that such procedures as injury to muscle or abdominal manipulation produce a rapid decline in the blood pressure and death of adrenalectomized dogs, but do not do so in normal dogs unless the injury is carried to an extreme degree.

Here again there are marked differences in the preventive action of the various cortical steroids. Desoxycorticosterone is very effective in correcting the effects of muscle trauma, but quite ineffective against abdominal injuries. The corticosterone series and particularly 11-dehydro 17-hydroxycorticosterone are highly effective against both types of injury.

These observations have an important bearing on the etiology and possibly the treatment of traumatic and hemorrhagic shock but since this question lies outside the present discussion it will not be further pursued.

Resistance to toxic agents. The adrenalectomized animal is susceptible to an undue degree not only to physiological stresses but also to noxious agents which in comparable quantity produce only slight and temporary discomfort in normal animals. In two instances, that of histamine and typhoid vaccine, it has been found that protection was conferred by corticosterone but not by desoxycorticosterone. There are also reports that the liver damage caused in normal animals by carbon tetrachloride may be ameliorated by treatment with 11-dehydro 17-hydroxy corticosterone.

Muscle work. The old observation that the muscles of adrenalectomized animals are incapable of prolonged activity has been expanded by Ingle in recent years. This investigator has tested the ability of a large number of steroids to increase the working capacity of the muscles of adrenalectomized rats. In all his experiments the muscle has been worked *in situ* so there is the possibility that the early failure in untreated animals may at least in part be due to factors other than those in the muscle itself. Nevertheless these preparations have given very interesting and valuable information. It has been clearly shown that desoxycorticosterone and related compounds do not improve the working capacity of muscles while the members of the corticosterone series are highly effective. It should be remembered that the chemical changes associated with muscular contraction are of a complicated character and require carbohydrate not only for the contractile process itself but also a continued replenishment of this material by glucose derived from the liver. This, of course, is in addition to the maintenance of an adequate blood supply. Consequently, as Ingle and Lukens (7) have shown, the injection of glucose also increases to a considerable degree the performance of the muscles. Up to the present time there has not been any demonstration that the administration of cortical hormones will improve the working capacity of the muscles of normal animals.

Carbohydrate and protein metabolism. The known facts concerning the metabolism of carbohydrate and protein in adrenalectomized animals or those treated with cortical hormones are as follows.

a). Adrenalectomized animals maintained by the administration of sodium salts or by the use of desoxycorticosterone are exceedingly liable to develop hypoglycemia when exposed to circumstances in which the maintenance of the blood glucose level becomes dependent on the animals' own resources. Thus in some animals, particularly the rat and cat, fatal hypoglycemia may follow the withdrawal of food. This may also be shown in the dog although longer periods of fasting are required. The occurrence of hypoglycemia is greatly hastened if the animals are exposed to any procedure that accelerates carbohydrate utilization. Among these are exercise, exposure to cold and the administration of insulin. In all these instances it can be shown that the hypo-

glycemia is independent of any change in the electrolyte and water metabolism and, as mentioned above, is readily provoked in adrenalectomized animals maintained in good health with desoxycorticosterone.

The administration of cortical extract or the corticosterone compounds to these animals soon returns the blood glucose to normal even when food is withheld. In normal animals similar injections into fasting animals produce only a slight elevation of the blood glucose.

b). An examination of the carbohydrate levels of the liver and muscles of adrenalectomized animals indicates that as long as the animals are well fed and receiving an adequate supply of carbohydrate the liver and muscle glycogen are not unduly depleted although they are probably below normal. Britton states, however, that in his experiments they were always much reduced and indeed claims one of the features of such animals is their inability to form liver glycogen from glucose. There is general agreement that exposure to stress soon depletes the liver glycogen and as stated above this stress may be no more than the withdrawal of food for a period which would be easily tolerated by normal animals.

The behavior of the carbohydrate levels of adrenalectomized or normal animals injected with cortical hormones has yielded important information. It was first shown by Britton and Silvette that cortical extract not only elevated the blood glucose of adrenalectomized animals but also increased the liver glycogen. Similar though less decisive effects were also observed in normal animals. These results have been amply confirmed and extended. It is now known that the increase in liver glycogen is not at the expense of other carbohydrate stores and that there has occurred under the influence of the hormone an absolute increase in the carbohydrate content of the body which must in consequence have been derived from some non-carbohydrate precursor.

As in many other instances there is a marked difference in the capacity of various steroids to promote this formation of carbohydrate. The corticosterones are particularly effective but not compounds of the desoxycorticosterone type. Certain estrogens including diethylstilbestrol also increase the liver glycogen but much larger quantities are required (cf. below).

c). The absolute increase in the carbohydrate stores of fasted animals receiving a suitable cortical preparation is at the expense of the protein stores of the body. This is shown by the increased nitrogen excretion whose magnitude is always sufficient to account for the extra carbohydrate formed. In addition such animals always lose more weight than their untreated controls and so far there is no evidence of an increase in metabolic rate nor fat catabolism of sufficient magnitude to account for this difference.

d). In mice ingesting a diet rich in carbohydrate the injection of cortical extract or corticosterone is followed by remarkable increases in the liver glycogen and significant increases in the muscle glycogen. Under these circumstances the increase in urine nitrogen is much smaller and is in no way sufficient to account for the extra carbohydrate deposited as glycogen. In table 1 are shown some experiments carried out in this laboratory by Dr. B. Katzin in which considerable quantities of glucose were administered to previously fasted rats, one group of which received cortical extract at hourly intervals for 12 hours. For comparative purposes the effects of injection of crude anterior pituitary extract are also given.

It will be observed that rats treated with cortical extract deposit more liver glycogen than their controls and since the quantity of glucose laid down as muscle glycogen was also increased they must have oxidized less. This is also suggested by the increased quantity of glucose in the body fluids and the occurrence of glycosuria. Anterior pituitary extract produces a deposition of liver glycogen that is less than in un-

treated animals, but a well-marked increase in muscle glycogen. In addition, marked hyperglycemia and gross glycosuria occurred. If the quantities of glucose represented by muscle and liver glycogen formation and that remaining in the body fluids or appearing in the urine are subtracted from the total glucose absorbed, the difference very largely represents the proportion oxidized. The administration of cortical extract decreases this quantity to a significant degree but an even greater reduction is observed in rats that received anterior pituitary extract.

Recent experience has indicated that if, instead of cortical extracts, 11-dehydro 17-hydroxycorticosterone is injected much greater effects are produced. Indeed,

TABLE 1. EFFECT OF CORTICAL EXTRACT AND ANTERIOR PITUITARY EXTRACT ON THE DISPOSITION OF FED GLUCOSE IN THE RAT

All the animals were first fasted for 24 hours and then given three feedings of glucose at 4-hour intervals. The cortical extract[1] was given at the rate of 1 cc. an hour for 11 hours while the APE was given in the form of a crude saline extract at the start of the experiment and every four hours afterwards. The total period of observation was 12 hours. All the values are expressed in mg. per 100 gm. rat.

	Untreated	Cortical Extract	Anterior Pituitary Extract
Number of rats	10	9	11
Total glucose absorbed	2736	2877	2652
Absorption rate per hour	228	240	221
Deposited as liver glycogen	349	431	284
Deposited as muscle glycogen	384	577	512
As glucose in body fluids	26	41	123
As urine glucose	0	81	490
Oxidized (by difference)	1977	1747	1243
Urine nitrogen	27.8	33.2	24.0

Percentage Distribution of Absorbed Glucose

	As Liver Glycogen	As Muscle Glycogen	As Glucose in Body Fluids	As Urine Glucose	Oxidized (by difference)
Untreated	12.8	14.0	1.0	—	72.2
Cortical extract	15.0	20.0	1.4	2.8	60.8
Anterior pituitary extract	10.7	19.3	4.6	18.5	46.9

[1] Cortical extract supplied by the Upjohn Co., Kalamazoo, Mich.

Ingle (8) has recently found that the continual daily administration of this substance to rats fed a high carbohydrate diet results in severe hyperglycemia and glycosuria, although it could be shown that some other factor than a stimulation of gluconeogenesis must have been operative since the increased urinary nitrogen could only account for a fraction of the glucose present in the urine. In individuals suffering from Addison's disease Thorn and his colleagues have also shown that the corticosterone compounds significantly depress carbohydrate oxidation but desoxycorticosterone was without effect.

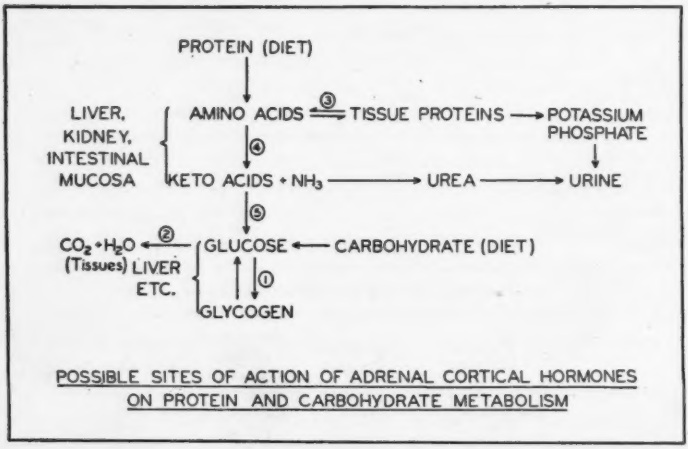
Prior to these experiments it had been shown in this laboratory that the diabetes of partially depancreatized rats could be exacerbated by injecting or feeding cortical extract or steroids of the corticosterone type. Desoxycorticosterone or progesterone were inactive. Gaunt and his colleagues also found certain estrogens to be diabetogenic in these preparations. It will also be recalled that adrenalectomy alleviates a pancreatic diabetes in cats, dogs and rats, as evidenced by the decreased urinary nitrogen and glucose excretion.

e). Adrenalectomized animals are very susceptible to the hypoglycemic action of insulin and even after small doses of this hormone exhibit a conspicuous failure to return spontaneously the blood glucose to normal. Insulin hypoglycemia in normal mice can also be prevented by the previous administration of corticosterone but not desoxycorticosterone (9).

Mechanism of Action of Cortical Hormones on Carbohydrate and Protein Metabolism

A perusal of the established facts derived from experiments on adrenalectomized animals or those injected with cortical hormones enables certain generalizations to be made regarding the influence of the adrenal cortex on carbohydrate and protein metabolism.

a). Adrenalectomized animals, while maintaining adequate carbohydrate levels so long as they are fed, fail to do so when placed under conditions that require them



POSSIBLE SITES OF ACTION OF ADRENAL CORTICAL HORMONES ON PROTEIN AND CARBOHYDRATE METABOLISM

to support these levels by the formation of glucose from non-carbohydrate sources (protein).

b). There is a marked qualitative difference between steroids of the type of desoxycorticosterone and those that possess an hydroxyl or carbonyl group at carbon 11. The former in nearly all the circumstances examined do not appear to influence carbohydrate or protein metabolism while the latter are highly active.

c). The conditions under which the corticosterones exert their influence are largely those in which the organism is forced to form glucose from protein. An excess of these hormones always accelerates protein catabolism under these conditions. Such circumstances are fasting, insulin hypoglycemia and pancreatic diabetes.

d). The administration of an excess of a corticosterone to animals receiving carbohydrate in their diet results in a decreased rate of carbohydrate oxidation and an excessive accumulation of glycogen in the liver and muscles.

Figure 2 shows the main transformations involving carbohydrate oxidation, glycogen formation and gluconeogenesis from protein. Alongside certain of these changes have been placed numbers which indicate the points at which various investigators have located the site of action of the cortical hormones.

Glycogen formation from glucose. Seckel (10) has reported that cortical extract inhibits glycogenolysis in surviving rat liver slices and concludes that one function of the adrenal cortex is to inhibit the glycogenolytic enzymes. This suggestion is an extension of the views of Britton and his colleagues who have long maintained that an inability to maintain adequate liver glycogen stores (and other carbohydrate levels) is a characteristic consequence of the loss of cortical hormone. Recently Britton and Corey (11) reported that insulin fails to induce glycogen storage in the liver and muscles of adrenalectomized cats given glucose. It is difficult to reconcile these observations with those of others who have found that salt-treated adrenalectomized rats and dogs will survive for long periods with approximately normal carbohydrate levels and when given glucose dispose of it in part by storage as glycogen (12). In further support of their views Corey and Britton (13) report that if cortical extract is perfused along with glucose through isolated rat liver that glycogenolysis is retarded. In the perfused cat liver when the cortical extract was added to a Ringer gum-glucose perfusate, an actual increase in the glycogen content was observed. Indeed, the authors state that a 100 per cent increase might occur in 10 or 15 minutes. Desoxycorticosterone was inactive and the addition of insulin did not cause an increase in liver glycogen. On the contrary, the usual result was a decrease.

If this view is correct then an excess of cortical hormone given to a normal animal leads to the accumulation of glycogen by preventing the breakdown of any originally present or formed either from dietary carbohydrate or non-carbohydrate precursors. Since the supply of carbohydrate to the tissues would be blocked by this effect on the liver glycogen they are forced to use more protein or fat for their energy requirements. In other words, a condition of internal carbohydrate lack which would result in many ways is akin to the clinical condition known as Von Gierke's disease.

Inhibition of carbohydrate oxidation. The recent work of Thorn and his colleagues (14), Ingle (8) and the experiments of Katzin quoted above (table 1) show that an excess of a suitable cortical hormone will inhibit glucose oxidation when ample carbohydrate is ingested. Since this occurs soon after injection it is unlikely that it is due to an interference with insulin secretion, although the ultimate occurrence of glycosuria after several days of injection may in part be due to this cause. In fasted animals, however, in which carbohydrate oxidation is already at a minimum, any further inhibition would seem incapable of exciting gluconeogenesis to the degree observed by several investigators and, indeed, all the experiments on fasted animals suggest a more direct effect of these hormones on carbohydrate formation from protein. In humans suffering from Addison's disease, Thorn et al. (14) have noted a high fasting respiratory quotient which rose to an abnormal degree after glucose administration. In adrenalectomized rats injected with glucose Evans (15) found an increased rate of disappearance of the sugar and suggested that this indicated an increased capacity to oxidize carbohydrate.

Acceleration of tissue protein catabolism. It has been demonstrated in several conditions that, as judged by the increased urinary nitrogen excretion, absence of cortical hormones retards tissue protein catabolism while an excess in normal animals accelerates this process. The breakdown of the tissue proteins liberates their constituent amino acids which after deamination yield residues which may form glucose or else be utilized without such a transformation.

The first point in the chain of protein catabolism that might be accelerated by cortical hormones is the conversion of the tissue proteins into amino acids. The site of this action might be confined exclusively to the peripheral tissues (muscles) or to the liver or to both.

No experiments have so far been reported in which a direct test of this possibility has been made.

Deamination of amino acids. The deamination of amino acids occurs largely if not entirely in the liver, kidney and intestinal mucosa, three tissues that incidently exhibit the most marked functional defects in adrenalectomized animals. Since in fasting animals the maintenance of the carbohydrate levels as well as the energy requirements of such essential organs as the kidney are almost entirely dependent on glucogenesis from this source any circumstance that reduced the rate of this process would result in their impairment. Russell and Wilhelmi (16) by measurement of the oxygen uptake and ammonia production have found that kidney slices from adrenalectomized rats do not deaminate such amino acids as alanine and glutamic acid at a normal rate. They have also reported (17) that in the kidney the rate of carbohydrate formation from these amino acids is reduced after adrenalectomy. Jiminez-Diez (18) also found a lowered deamination rate in the kidneys of adrenalectomized cats.

In liver slices either from normal or adrenalectomized rats Koepf et al. (19), found no difference in the carbohydrate formation from glutamic acid. Alanine did not form carbohydrate in either tissue. This would indicate a difference between the behavior of the liver and kidney in this regard.

In adrenalectomized rats Samuels et al. (20), have reported that the rate of of removal of fed alanine from the blood as well as the formation of liver glycogen from this amino acid was decreased. However, Evans (21) has found in adrenalectomized and functionally nephrectomized rats that the rate of disappearance of injected alanine was not different from normal rats and deamination as judged by urea formation was also normal.

Conversion of α-keto and α-hydroxy acids to glucose. Buell, Anderson and Strauss (22) reported some years ago that the livers of adrenalectomized rats were unable to convert fed lactic acid into liver glycogen at a normal rate. Tipton (23) later showed that liver slices from such animals had a decreased ability to oxidize pyruvate and succinate, a finding later confirmed by Russell and Wilhelmi (16) in the kidney. Although these authors (17) have reported that in the kidney carbohydrate formation from pyruvate and succinate is not impaired, Koepf et al., state that liver slices taken from adrenalectomized animals have a reduced capacity to form carbohydrate from pyruvate. Previous treatment of either normal or adrenalectomized rats with cortical extract increased the carbohydrate formation. They were, however, unable to detect any difference in the ability of the tissues to form carbohydrate from lactate. Previous treatment of normal or adrenalectomized animals with cortical extract also increased carbohydrate formation to an equal degree.

Lewis and his colleagues (24) injected lactic, pyruvic acid and alanine into phloridzinized rats and by use of the urinary G:N ratio calculated that after adrenalectomy only 20 per cent of glucose was formed from lactic acid as compared to 74 per cent in normal animals. The figures for pyruvic acid were 34 per cent and 102 per cent and for alanine 57 and 90 per cent. They conclude that the defect in the gluconeogenetic processes in adrenalectomized animals to be an inability to convert the amino acid residues into glucose.

It should be noted that Russell and Wilhelmi (17) found that α-keto-glutaric acid formed significantly less carbohydrate in the kidney slices of adrenalectomized rats and suggest that even in this organ the rate of gluconeogenesis may be retarded as much by the failure to utilize the amino acid residues as by a retardation of deamination itself.

Up to the present time the results of experiments assigned to test the capacity of individual tissues to metabolize carbohydrate and protein in the presence or absence of the cortical hormone along with those obtained on the whole animal suggest that the hormone exerts its influence at two points: *a*), on the capacity of the peripheral tissues to utilize glucose, and *b*), on the formation of carbohydrate from smaller molecules, particularly in the liver and kidney. There is evidence that either the rate of deamination by which glucogenic carbon residues are formed, or the actual condensation of these residues to form glucose is impaired by adrenalectomy and enhanced by an excess of cortical hormones. It may also be assumed that these effects are related to steroids of the type of corticosterone since so far as it has been tested desoxycorticosterone is inactive.

Relation Between the Effects on the Organic and Inorganic Metabolism

One of the most puzzling features of the action of the adrenal cortex is the apparent dissociation between its effects on the organic and inorganic metabolism. This has become particularly evident since it has been shown that cortical extracts can be divided into components that influence one without affecting the other (25) as well as by the separation of steroids with the same diversity of action.

On the other hand, corticosterone not only controls the carbohydrate and protein metabolism but will maintain the electrolyte balance of adrenalectomized animals. Here evidently is a representative of a group of closely allied compounds specific to the adrenal cortex which are capable of repairing all the disabilities caused by adrenalectomy. This in my opinion necessitates the conclusion that these steroids are the essential and characteristic hormones of the adrenal cortex.

Desoxycorticosterone although it also is found only in the adrenal cortex does not represent a substance capable of correcting all the deficiencies of adrenalectomized animals. True it exerts a powerful effect on electrolyte metabolism, greater indeed than the corticosterones but it is singularly incapable of correcting the disturbed organic metabolism. Furthermore, other substances that are not typical adrenal derivatives do, at least in normal animals, show a capacity to distort the electrolyte metabolism and one of them, progesterone, shares to a minor degree the ability of desoxycorticosterone to maintain life.

A further complication has recently been introduced by Thorn and his associates (26), who point out that those corticosterones possessing an hydroxyl group at carbon 17 augment the sodium and chloride excretion of normal dogs and rats. This we can confirm from our recent experiments on fasted rats but it will be recalled that these compounds are those that possess the most marked effect on carbohydrate and protein metabolism and, indeed, their injection is always accompanied by marked increases in the urinary nitrogen, potassium and phosphate.

It is reasonable to suppose that the increased potassium and phosphate excretion in animals treated with corticosterone is derived, along with the urine nitrogen, from the same source, that is the tissues, since it is known that any agent that increases the rate of tissue breakdown will liberate the inorganic salts associated with the cells (cf. fig. 1). However, desoxycorticosterone also increases potassium excretion but in this case it is found that the serum potassium is reduced, while the injection of corticosterones either leaves the level unchanged or slightly decreased.

Nevertheless, the striking effect of desoxycorticosterone on electrolyte and water metabolism without a comparable effect on carbohydrate and protein metabolism is a stumbling block to an unified hypothesis of adrenal cortical function. For if these two effects were always related it would become possible to suggest that the hor-

mones of this gland controlled the rate of some chemical transformation upon which certain of the organs of the body depended for their function. The deficiency of these hormones would then be expressed by these organs in accordance with their function; in the kidney by an inability to supply energy at a sufficiently high rate for the maintenance of the electrolyte metabolism, in the muscles in an incapacity to do work, and in the liver by a comparative failure of those mechanisms upon which the maintenance of a normal carbohydrate metabolism depends.

The 'Corticomimetic' Action of Estrogens

It has already been pointed out that some estrogens, including diethylstilbestrol, are able to reproduce in normal animals certain changes in the organic and inorganic metabolism that have come to be associated with the cortical hormones. Thus, Thorn and Engel (3) found them to induce a retention of sodium and chloride and

TABLE 2. EFFECT OF STILBESTROL (5 MG. PER 100 GM.) ON THE CARBOHYDRATE LEVELS AND NITROGEN EXCRETION OF NORMAL, ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS

All values expressed as mg./100 gm. of rat. Period of observation, 30 hours. Total fast, 54 hours.

	Number of Rats	Glycogen		Glucose of Body Fluids	Urine Nitrogen
		Liver	Muscles		
Normal controls	15	27.7	270	49.5	100±3.7
Normal+Diethylstilbestrol	7	56.0	276	52.0	114±5.5
Adrenectomized Controls	4	1.1	200	32.0	81±8.3
Adrenectomized+Compound *E*[1]	4	71.9	270	48.5	144
Adrenectomized+Diethylstilbestrol	7	4.1	205	29.5	89±5.3
Hypophysectomized Controls	1	1.7	137	39.0	137
Hypophysectomized+Diethylstilbestrol	3	1.2	124	36.0	124

[1] Compound *E* is 11-dehydro 17-hydroxycorticosterone. 1.5 mg. per 100 gm. was given in oil.

an increased excretion of potassium in the dog while Janes and Nelson (27), as well as Griffith, Marks and Young report that they increase the liver glycogen of fasted rats. The former also observed an increase in the nitrogen excretion after treatment with diethylstilbestrol.

Dolin, Joseph and Gaunt (28) found estrogens to augment the glycosuria of partially depancreatized ferrets while Ingle (29) has found that continued diethylstilbestrol injection will produce glycosuria in normal rats fed a diet rich in carbohydrate.

The estrogens also increase the insulin content of the pancreas but recently Fraenkel-Contrat et al. (30), have shown that this is dependent on the presence of the hypophysis since they were unable to detect such a change in the insulin content of the pancreas of hypophysectomized rats. Furthermore, it is stated in this paper that the liver glycogen was not increased in these animals.

Now it is well-known that the estrogens, particularly diethylstilbestrol, cause great enlargement of the adrenal cortex which in turn is dependent on the stimulation of the anterior pituitary. Consequently, it would appear that at least so far as the effects of these substances on carbohydrate and protein metabolism is concerned that it is exerted through this mechanism and is in reality an expression of increased cortical activity.

In this laboratory this hypothesis has recently been tested in the following manner.

Rats were either adrenalectomized or hypophysectomized[2] and immediately

[2] We are indebted to Drs. J. A. Russell and A. E. Wilhelmi for performing the hypophysectomies.

fasted. Twenty-four hours later they were injected with 5 mg. per 100 gm. of diethylstilbestrol dissolved in 0.5 cc. of sesame oil, placed in metabolism cages and the urine collected over the next 30 hours. During this time the adrenalectomized animals received several intraperitoneal injections of sodium chloride. At the end of the period they were anesthetized and samples of liver muscle and blood removed for analysis. Thee rsults are shown in table 2, where the operated animals are compared to normal animals receiving diethylstilbestrol and to adrenalectomized animals receiving 1.5 mg. per 100 gm. of 11-dehydro 17-hydroxycorticosterone.

It will be observed (table 2) that in confirmation of the work quoted above the diethylstilbestrol-treated normal animals have an increased liver glycogen and urinary nitrogen excretion. The blood glucose and muscle glycogen are not different from those of the untreated normal animals.

The adrenalectomized untreated animals have a decreased urinary nitrogen excretion and a very low liver glycogen. The blood glucose level is also decreased while the muscle glycogen content is below that of the normal controls.

The injection of diethylstilbestrol did not elevate either the urine nitrogen, the liver muscle glycogen or the blood glucose. In marked contrast to this are the striking changes produced by the much smaller quantities of 11-dehydro 17-hydroxycorticosterone. This hormone increased the urine nitrogen and liver glycogen to levels greater than in normal animals, while the muscle glycogen and blood glucose were increased to about the levels encountered in the normal controls.

The failure of diethylstilbestrol to influence the carbohydrate and protein metabolism would seem to indicate clearly that its positive effect in normal animals is due to its secondary action on the adrenal cortex.

In a smaller number of experiments the effect of diethylstilbestrol on freshly hypophysectomized rats was studied. Only one control animal is cited which exhibits the characteristic low carbohydrate levels of these animals. The high level of nitrogen excretion of this animal which far exceeds that of normal animals is, in our experience, always observed in freshly hypophysectomized and fasted rats. It is undoubtedly due to the sudden withdrawal of that pituitary agent (growth hormone?) that regulates protein anabolism and this high level of nitrogen excretion is in marked contrast to the decreased excretion of freshly adrenalectomized rats.

Nevertheless, in spite of the increased protein breakdown present in these animals the injection of diethylstilbestrol failed to cause any accumulation of liver glycogen nor did it elevate the blood glucose or muscle glycogen. The excretion of nitrogen was not further increased by the injection. In previous experiments we have shown that the injection of cortical extract or corticosterone will increase the nitrogen excretion and the carbohydrate levels of fasted hypophysectomized rats.

It would appear therefore that the effect of estrogens is due to their influence on the anterior pituitary and the adrenal cortex and since the secretory activity of the latter is dependent on the former in the last analyses it is the action of the estrogen on the anterior pituitary that is the determining factor.

The question now arises as to whether the influence of estrogens on the electrolyte and water metabolism is not also due to the same cause. In this regard it should be recalled that in spite of their salt-retaining capacity in normal animals, the estrogens do not maintain the life of those deprived of their adrenals. To the contrary, several investigators have reported a deleterious action.

Gaunt, Nelson and Loomis (31) found that the estrogens did not prevent the occurrence of fatal water intoxication in adrenalectomized rats although protection was conferred by cortical extract.

In their studies on the effect of sex hormones on the renal excretion of electrolytes in dogs Thorn and Engel (3) found what they term a 'partial prevention' of the excessive sodium-chloride loss that follows withdrawal of extract in two adrenalectomized animals when a mixture of the natural estrogens was injected after discontinuance of the cortical extract.

The question appears worthy of re-examination although it is not easy to design satisfactory experiments since this corticomimetic action of estrogens has presented one of the most puzzling problems in this already complicated field.

In the experiments reported above the urinary excretion of potassium and chloride was determined in the different groups of animals. No effect of diethylstilbestrol on the urinary excretion could be detected even in the normal animals so it is evident that other types of experiments must be arranged to settle this point. Furthermore, the adrenalectomized rat does not exhibit the high rate of electrolyte loss that is so characteristic of the adrenalectomized dog.

It may, however, be concluded that it has been shown that the ability of estrogens to influence the carbohydrate and protein metabolism does depend on the presence of the adrenal cortex and anterior pituitary. Furthermore, it has also been shown that their capacity to increase the insulin content of the pancreas is also dependent on the presence of the hypophysis and in view of the close connection between these events probably also upon the ability of the adrenal cortex to respond to the hypophyseal stimulus.

SUMMARY

1. The main facts concerning the influence of cortical hormones on the inorganic and organic metabolism are outlined.

2. The exact mechanism of action of the cortical hormones on carbohydrate and protein metabolism is not yet understood but it is suggested that two processes seem to be influenced by them: *a*), the oxidation of glucose in the peripheral tissues; *b*), the formation of carbohydrate from amino acids or their residues after deamination.

3. The changes in the inorganic and organic metabolism induced by cortical hormones are not at present capable of interpretation in terms of any one hypothesis, although it is pointed out that certain alterations in the former apparently are due to disturbances in the latter. Among these are the increased potassium and phosphate excretion that accompany the increased rate of gluconeogenesis.

4. Experiments are reported which indicate that the action of estrogens in increasing the liver glycogen and nitrogen excretion of fasted rats is due to their stimulation of the adrenal cortex by their action on the anterior pituitary, since they do not produce these effects in adrenalectomized and hypophysectomized animals.

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ADVANCEMENT OF KNOWLEDGE OF THE RÔLE OF THE HYPOPHYSIS IN CARBOHYDRATE METABOLISM DURING THE LAST TWENTY-FIVE YEARS

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IN THE LAST TWENTY-FIVE YEARS the knowledge of the part played by the hypophysis in carbohydrate metabolism has passed through two stages. In the first, from 1908 to 1929, all of the actions were attributed to the posterior lobe; in the second, from 1929 and 1930, the importance of the anterior lobe was discovered, since its extract was found to be more potent than posterior lobe extract and it can produce a diabetic condition. At the same time definite proof was obtained of the physiological significance of the anterior hypophysis in the regulation of carbohydrate metabolism in the normal state and in diabetes.

Investigations Prior to 1929

During the first period the following facts were established: *a*), frequency of the association of glycosuria and diabetes in acromegaly; *b*), capacity of posterior lobe extracts to produce hyperglycemia and glycosuria; *c*), the antagonism between the action of insulin and posterior lobe extract; *d*), the sensitivity of hypophysectomized animals to insulin; *e*) the tolerance of hypophysectomized animals for different sugars.

Glycosuria and diabetes in acromegaly. Since Pierre Marie, the discoverer of acromegaly, reported the great frequency of glycosuria in this disease, various papers have been published confirming this statement. The figures given in two of the largest statistical summaries show that of 176 cases, 71 (40%),[1] and of 817 cases, 268 (33%),[2] had glycosuria.

Hyperglycemia and glycosuria produced by posterior lobe extract. The fact that glycosuria occurs frequently in acromegaly led Borchardt (12), in 1908, to inject hypophyseal extract into rabbits; glycosuria appeared in 2 to 6 hours after the injection and lasted for 24 hours. In the dog glycosuria was not always observed. Borchardt deduced that glycosuria in acromegaly was due to an increase in hypophyseal secretion. Based on these facts, Brugsch (14), in 1916, postulated that diabetic glycosuria could be due to: *a*), pancreatic deficiency (insular type); *b*), intense hypophyseal stimulation (hypophyseal type); *c*), both factors acting simultaneously (mixed or usual type).

Glycosuria is due to hyperglycemia, as was shown by Borchardt (12) and later studied by many workers. Houssay and di Benedeto (57) observed that hyperglycemia is not produced by posterior lobe extracts after the liver or the adrenals has been removed. Hyperglycemia is not due to the discharge of epinephrin by the adrenals, but it does not occur if the normal secretion of epinephrin is lacking. The posterior lobe extract produces hypoglycemia in adrenalectomized animals, but this is not due to an increased insulin secretion, since it also occurs after pancreatectomy.

[1] Borchardt, L.: *Zeit. f. klin. Med.* 66: 332. 1908.
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Antagonism between insulin and posterior lobe extract. This antagonism has been shown to exist in respect to the toxic reaction, the levels of blood sugar and other blood components, fatty infiltration of the liver, diuresis, tissue imbibition, arterial blood pressure, respiratory exchange and skin color in batrachians. A summary of work done on the subject has been given by Houssay (38).

Posterior lobe extract can increase the blood sugar and suppress hypoglycemic symptoms produced by insulin, as was shown in 1923 by Burn (15) in the rabbit and by Magenta and Biasotti (82) in the dog.

Sensitivity of hypophysectomized animals to insulin. Marked hypersensitivity to insulin was first seen in hypophysectomized animals and studied from 1924 to 1929 by Houssay and Magenta (64, 65, 66). Small doses, innocuous to normal animals, produce in the hypophysectomized animal a pronounced hypoglycemia, convulsions and frequently death; when the animals survive, recovery is very slow. Early injection of glucose diminishes the severity of the symptoms. No increased sensitiveness to insulin follows lesions of the *tuber cinereum* in the area behind the hypophyseal stalk. The oxytocic principle of the posterior lobe is more active than the hypertensive principle in antagonizing the convulsive and toxic effect of insulin.

Three years after our first paper, Geiling, Campbell and Ishikawa (35) confirmed our findings, but erroneously attributed the hypersensitivity to the deficiency of the posterior lobe. Since then hypersensitiveness to insulin following hypophysectomy has been seen in many species (39). It has been proved to be due to the removal of the anterior lobe and it can be annulled by the previous injection of extracts of the anterior hypophysis.

Tolerance to sugars in hypophysectomized animals. The work of Cushing must be considered a forerunner in this field, since he studied the rôle of the hypophysis in carbohydrate metabolism from 1911 to 1927. In his papers with Goetsch and Jacobson (36) and Weed and Jacobson (104) he remarked that hypophysectomized dogs have an increased tolerance to sugar, since they can be given large doses of saccharose without developing glycosuria.[3] This increase in tolerance was also observed in the hypophysectomized dogs after removal of large portions of the pancreas, but total pancreatectomy was not performed, nor was the blood sugar examined. The increased tolerance was attributed to the posterior lobe because it was observed after removal of this lobe and because posterior lobe extracts diminished the sugar tolerance in normal and in hypophysectomized dogs. In 1913 Weed, Cushing and Jacobson (104) attributed a glycogenolytic and glycosuric hormone to the hypophysis, the secretion of which could be produced by nervous impulses sent to the gland through the superior cervical ganglion. Later in 1930, Cushing (24) thought that this glycogenolytic action was originated more probably in the hypothalamus than in the hypophysis.

Investigations Since 1929

In 1929 and 1930 the rôle of the anterior lobe of the hypophysis in the regulation of carbohydrate metabolism was proved, both in the normal and the diabetic states. The proof obtained in chronological order was the following. *a*), Hypersensitivity to insulin of hypophysectomized animals is due to loss of the anterior and not the posterior lobe. *b*), Anterior hypophyseal extract increases resistance to insulin. *c*), Hypophysectomized animals easily fall into hypoglycemia and lose their hepatic glycogen; this is prevented by giving anterior lobe extract. *d*), Hypophysectomy or extirpation of

[3] This might be due to a slower absorption of the sugar, as occurs in hypophysectomized rats (87), but this does not occur in toads. Intravenous injection of glucose produces a more prolonged hyperglycemia in hypophysectomized dogs (9, 34) and rats (4, 97).

the anterior lobe attenuates diabetes (pancreatic and phloridzinic) *e*), Anterior lobe extract has a diabetogenic action.

Hypersensitivity to insulin after hypophysectomy is due to the loss of the anterior lobe. In 1925 Houssay, Mazzocco and Rietti (67), and in 1929 Houssay and Potick (68) found that extirpation of the anterior lobe only of the hypophysis increased the sensitivity to insulin. This work was later extended to the rat in 1936 by Pencharz, Cori and Russell (86) and Swann and Fitzgerald (102, 103) in 1938. Removal of the posterior lobe alone does not modify the reaction to insulin (72, 86). Therefore the part played by the hypophysis in maintaining a normal resistance to insulin is due only to the anterior lobe.

Decisive proof of this was given by Houssay and Potick (68) in the paper already mentioned by demonstrating that hypersensitivity to the toxic and hypoglycemic action of insulin could be more easily corrected with the anterior lobe of the toad or bovine hypophysis, than with the posterior lobe. This was also seen in the normal dog in 1932 by di Benedetto (27) and in 1936 by Houssay and Foglia (59), and in the normal rabbit in 1934 by Cope and Marks (23), who published an important paper on the subject. This anti-insulin effect was called glycotropic activity by Young (106, 107) who has given proof that it is not due to the diabetogenic, thyrotropic, gonadotropic, ketogenic, melanophore-expanding substances, or to prolactin. Recently it has been maintained that the glycotropic is the same as the adrenotropic substance, but there is some evidence against this opinion.

In hypophyseal cachexia (Simmonds' disease), hypersensitivity to insulin has been repeatedly encountered (39). On the other hand Davidoff and Cushing (25) reported in 1927 that in acromegalic diabetes larger doses of insulin are necessary to control the blood sugar than in other forms of diabetes. They attributed this insulin resistance to hyperactivity of the hypophysis, since partial removal of the adenoma reestablished a normal response to insulin. Further observations have shown that in some cases of acromegaly there is a marked resistance to insulin, while in others the response is normal. This difference can be explained by the fact that insulin resistance occurs only while there is an excess of anterior hypophyseal hormone in the circulation; later a permanent diabetes may be established by the existence of irreversible lesions in the islets of the pancreas, in spite of there being no longer an excess of hypophyseal hormone.

In hypophyseal insufficiency many factors easily produce hypoglycemia: *a*), fasting (13, 43, 45); *b*), postoperative cachexia; *c*), phloridzin and insulin; and *d*), it may occur secondarily to the hyperglycemia due to epinephrin or glucose administration (cf. 39 for bibliography). The 'spontaneous' occurrence of hypoglycemic crises was seen in man by Wilder (105) in 1929, and the following year by Houssay and Biasotti (43) in hypophysectomized dogs and in dogs in which both the hypophysis and the pancreas had been removed. Later this finding has been confirmed and extended to other species by many observers (39). The hypoglycemia seen in fasting hypophysectomized animals is remarkable; it produces convulsions and may be lethal. Timely injection of glucose makes it disappear at once (the injection should not be made too rapidly, otherwise it may kill the animal). The protective and curative actions of epinephrin and posterior hypophyseal extract are less sure. After giving glucose it is necessary to feed the animals, otherwise relapses, sometimes fatal, may occur.

In hypophyseal insufficiency phloridzin produces in fasting dogs a fatal hypoglycemia (11, 44, 48, 54); the same observation has been made in toads (26). A diet rich in protein or carbohydrate prevents hypoglycemia produced by phloridzin (11, 48) and by fasting (101); a fatty diet does not protect the animal.

Substances and agents that produce hyperglycemia cause a subnormal, normal or supernormal increase in blood sugar in hypophysectomized animals according to the substance or agent used, the way in which it is given and the nutritional condition of the animal (13, 28, 39, 45, 94). Intravenous injection of glucose is followed by a rise in blood sugar which is more prolonged than in controls (9, 13, 39, 97).

Anterior hypophysis and glycogen. Hypophysectomized animals, shortly after having been fed, have normal quantities of glycogen in the liver and muscle, but there is a rapid fall during fasting. This fact has been observed in the toad (58), the dog (39), the rat (94, 95, 96) and the rabbit (22). In the toad the heart glycogen also diminishes (85). Russell (93, 94) maintains that the drop in muscle glycogen is characteristic of hypophyseal insufficiency and can be prevented by giving anterior lobe, which apparently has a glycostatic factor that can act even after adrenalectomy (94, 95, 96). This specific decrease in muscle glycogen has been seen only in the rat; it has not been demonstrated in other animals (39) and no proof has been given that the glycostatic factor differs from the glycotropic factor (111).

Attenuation of diabetes in anterior hypophyseal insufficiency. The remarkable decrease in the severity of the symptoms of pancreatic diabetes following total hypophysectomy, or extirpation of the anterior lobe, was discovered in 1929 and 1930 by Houssay and Biasotti (42) in the toad *Bufo Arenarum Hensel.* The increase in blood sugar was found to be considerably less than in the controls, and glycosuria did not occur. They also showed that subcutaneous implantation of anterior lobe reestablishes the intensity of the diabetes or may even increase it above that seen in the controls. This was the first demonstration of a physiological and pharmacological diabetogenic activity of the anterior hypophysis.

This same attenuation of pancreatic diabetes by hypophysectomy was confirmed in the dog by Houssay and Biasotti (38, 39, 43, 45) and by many others afterwards (6, 21, 39, 94, 99) in mammals, reptiles, amphibia and fishes (39). The hypophysectomized-pancreatectomized dog shows the following differences when compared with the simply pancreatectomized dog. Life is prolonged, in some cases to 6 and even 9 months; wound healing is almost normal and infections are less frequent; loss of weight is more gradual; hyperglycemia and glycosuria are less marked or may not occur; during fasting they diminish significantly; 'spontaneous' hypoglycemic crises may occur in underfed or fasting animals; there is great sensitivity to insulin, which easily produces hypoglycemic crises; these crises can be stopped by the early administration of glucose; muscle and liver glycogen decrease only a little and may remain normal if the diabetes is not severe; when glucose is given only part of it is eliminated in the urine; in some cases it is totally retained (43, 45); the hyperglycemic curve is intermediate between the normal and diabetic one (6, 10, 21, 99); the respiratory quotient increases somewhat after giving glucose, but in some cases this does not occur (10, 21, 39, 45); ketonemia and ketonuria show little increase (91, 92); the alkaline reserve is almost normal; calcemia is slightly decreased (there is hyperproteinemia, and disseminated lesions in the parathyroid glands have been reported); lipemia and cholesterolemia increase less; the fasting urinary nitrogen excretion is diminished; the increase in basal metabolism is less marked; the D:N ratio is lower. Further details will be found in Houssay's papers (39, 40).

Hypophysectomy also attenuates phloridzin diabetes in dogs; glycosuria, urinary nitrogen and ketonuria diminish. The administration of anterior lobe extract intensifies these attenuated symptoms as was demonstrated by Houssay, Biasotti and their collaborators (11, 26, 44, 48, 54). Extirpation of the posterior lobe does not attenuate pancreatic or phloridzin diabetes in the dog (11, 44, 48, 54).

The activity of the anterior hypophysis is a physiological factor that increases the severity of all forms of diabetes. Diabetes is a disturbance in the endocrine equilibrium between the secretion of insulin on one hand and the secretions of the anterior hypophysis, adrenals and thyroid on the other. This equilibrium regulates the production of sugar by the liver and its utilization by the tissues, especially the muscles.

Diabetogenic action of anterior hypophyseal extract. The diabetogenic activity of the anterior hypophysis was discovered in 1930 by Houssay and Biasotti (42) when they found that implantation of the anterior hypophysis, or injection of its extract reestablished the severity of diabetes, or even enhanced it, in the hypophysectomized-pancreatectomized toad. Later (1932) this was confirmed in the dog (53, 55) and in other species (39, 40, 45, 46). The diabetogenic activity was demonstrated in: *a*), dogs with subtotal pancreatectomy and normal blood sugar (53, 51, 61); *b*), dogs suffering a Sandmeyer's diabetes (53, 55); and *c*), dogs with intact pancreas (39, 53, 55). As the amount of pancreatic tissue is reduced smaller quantities of anterior hypophysis are necessary to produce diabetes (53, 55, 61). In pancreatectomized dogs injection of the extract considerably increases the severity of diabetes, with intense ketosis, acidosis, coma and death in one to three days (39, 40).

The diabetogenic action of the anterior hypophysis was first demonstrated in batrachians in 1930 and in mammals in 1932, but before this, in 1927, Johns et al. (71) reported than an extract, the preparation of which was not described but which was stated to be 'protein free,' produced moderate hyperglycemia. According to our observations the diabetogenic factor is a protein.

The diabetogenic action was demonstrated in normal mammals in 1932, by Evans, Meyer, Simpson and Reichert (31), Bauman and Marine (5) and Houssay, Biasotti and Rietti (53, 55); in 1933 by Barnes and Regan (6) and E. Evans (30). Since 1932 it has been studied in our Institute without interruption in more than 800 dogs. In 1936 I visited the United States and Canada and found that it could not be demonstrated in the laboratories of Evans (94), Collip, Long and others; this had given rise to certain doubts as to the correctness of previous observations. The difficulty was due to the fact that the diabetogenic activity of the tissue is lost at room temperature so that it is necessary to put the glands on ice as soon as they are removed from the animal and to keep the extracts at approximately 0°C. In 1935 I had informed Young of this peculiarity of the extract and he was able to obtain potent preparations, with which he made important observations (107–111).

In the dog, a daily intraperitoneal injection of extract produces a rise in blood sugar on the 2nd or 3rd day, which eventually reaches a fasting level of 150 to 300 mg. per cent. The average figure in 300 dogs has been 178 mg. per cent. During the day slight variations can be seen. Some dogs are resistant and have little or no hyperglycemia. The increase is not considerable and may not occur if the animals are kept fasting; a carbohydrate diet on the other hand favors a rapid and intense rise. When the treatment is continued the blood sugar remains high for 3 to 7 days; after this it may decrease (53, 55, 107) and even fall to subnormal levels (30); when it decreases a larger dose produces a further increase in blood sugar (107). Whether the blood sugar rises or stays normal there is a characteristic increase in resistance to insulin, both to that injected and to that secreted by a grafted pancreas, in all animals with a diabetes of hypophyseal origin (59).

The symptoms of this diabetes have been described by us in several papers (39, 40, 45, 55, 56) and later by Young (107–111). Characteristic points are the following: *a*), the increase in blood sugar does not begin till the 2nd or 3rd day; *b*), it does not occur if the animals are kept fasting; *c*), when the injections are discontinued

the blood sugar decreases rapidly and in 1 to 4 days is again normal; *d*), the liver glycogen is normal or only slightly decreased (this is the only type of diabetes in which this occurs); *e*), there is a typical increase in resistance to insulin; *f*), diabetes still occurs after removal of thyroid and adrenals, but not after hepatectomy.

Hyperglycemia and glycosuria, increase of ketone bodies in blood and urine (91, 92), polydipsia and polyuria, increase in blood fat and cholesterol, and in liver fat to three times the normal value, an increase in plasma protein, and a decrease in plasma chlorine, sodium and the alkaline reserve are prominent symptoms. Injection of glucose produces a prolonged hyperglycemic curve, which approaches that seen in pancreatic diabetes (6, 8, 30); the sugar is in great part eliminated in the urine; the respiratory quotient does not rise in animals that have a high fasting blood sugar.

The diabetogenic action has no zoologic specificity since the hypophysis of fishes, batrachians, birds and mammals is active in batrachians and mammals, as has been shown by us since 1930 (42, 46, 47, 55, 56) and confirmed by others (6, 30, 107). The relative potency in diabetogenic activity of different species has been determined (69).

Several chemical properties of the diabetogenic substance have been studied by Houssay and his collaborators, from 1930 and 1934 onward (39, 40, 42, 46, 55, 56) and by Young in 1938 (107, 111) but up to now it has not been identified as a chemical entity, nor can we be sure it is only one substance. It has been proved that it is not any one of the following hormones: thyrotropic, adrenotropic, gonadotropic, glycotropic, growth hormone and prolactin (51, 107, 111). I have never spoken of a 'diabetogenic hormone' which is an improper term, but of a diabetogenic factor, activity or property. This hormonal substance (or substances) regulates carbohydrate metabolism, but its physiological rôle is certainly not the causation of diabetes. Nevertheless, the physiological function of the anterior hypophysis is necessary for the establishment of a severe diabetes.

Several different effects of anterior lobe extracts on carbohydrate metabolism have been reported: *a*), the diabetogenic, by Houssay and Biasotti (42, 46, 53–56) in 1930; *b*), the contra-insular, by Lucke (80, 81) in 1934; *c*), the pancreatotropic, by Anselmino, Hoffmann and Herold (1, 2) in 1933; *d*), the glycogenolytic (Kohlenhydratstoffwechsel-hormon), by Anselmino and Hoffman; *e*), the hyperglycemic ultrafiltratable factor, by Anselmino and Hoffmann in 1935; *f*), Collip's specific factor, which he describes as glycostatic, ketogenic and a depressor of the respiratory quotient. With the exception of the first of these, it has not been proved that any of these substances is secreted by the gland, nor that they have a physiological rôle. The contra-insular activity is probably due to contamination with posterior lobe substances, since the slight, transitory and inconstant hyperglycemia it produces is not observed after removal of the adrenal medulla or section of the splanchnic nerves; neither does it have a diabetogenic action according to Houssay and Biasotti (51).

It is incorrect to call the diabetogenic substance a contra-insular hormone because its principal activity is extrapancreatic; on the other hand several hormones have contra-insular activity (epinephrin and posterior hypophysis). The hypophyseal diabetogenic activity should not be identified with the rapid and transitory hyperglycemic activity of several hypophyseal and urinary extracts of doubtful specificity and no proven physiological significance.

Rôle of the liver. The diabetogenic action is dependent on the liver; hepatectomy suppresses the diabetogenic effect of the anterior hypophysis in pancreatectomized-hypophysectomized toads (Campos, Curutchet and Lanari, 19; Foglia, 33). When the mass of the liver is reduced gradually and the extirpation surpasses 55 per cent of the total weight the diabetogenic action diminishes progressively in proportion to the

mass removed (31). In the dog with an hypophyseal diabetes hepatectomy is followed by a rapidly fatal hypoglycemia, which can be strikingly although temporarily ameliorated by the injection of glucose (59). The part played by the anterior hypophysis in the regulation of liver function will be discussed later.

Neurohypophysis and nervous system. Posterior lobe extract produces hyperglycemia of short duration; a diabetic state cannot be produced by repeated injections or prolonged treatment in a normal animal. This extract has certain pharmacological effects on carbohydrate metabolism: *a*), increase in the resistance to insulin (15, 39, 68, 82); *b*), increase in the blood sugar of hypophysectomized-pancreatectomized animals (42); *c*), improvement of asthenia of hypophysectomized toads, and *c*), slight increase in the liver glycogen (58). All of these effects are less marked than those produced by the anterior lobe extract. The physiological importance of the posterior hypophysis in carbohydrate metabolism apparently has little significance, since the removal of only the anterior lobe is followed by all the metabolic disturbances, asthenia, increased sensitivity to insulin and attenuation of pancreatic diabetes that occur after total hypophysectomy (42). On the other hand, the removal of the posterior lobe in mammals does not increase sensitivity to insulin (80, 102) and does not modify the severity of pancreatic or phloridzin diabetes (11, 42, 45, 48).

The diabetogenic action of the anterior hypophysis can be demonstrated in the hypophysectomized-pancreatectomized toad after removal of the anterior and intermediate hypophysis and midbrain. The participation of the hypothalamus in diabetes and in carbohydrate metabolism is still an open question (see bibliography and discussion in Houssay, 39 and Long, 77).

Other organs. In the hypophysectomized-pancreatectomized toad the diabetogenic effect of the anterior hypophysis can be obtained after extirpation of the digestive tract, lungs, kidneys, thyroid, testicles and ovaries (46, 47). It can also be obtained in dogs after removal of the gonads, thyroid, adrenal medulla and abdominal sympathetic chains (Houssay, Biasotti and Rietti, 56).

Rôle of the pancreas. Pancreatic and extrapancreatic factors play a part in anterior hypophyseal diabetes. The extrapancreatic factors act before the islets have been damaged sufficiently to decrease the secretion of insulin (60). The existence of extrapancreatic factors is proved by the following facts. *a*), Diabetogenic activity can be demonstrated in the absence of the pancreas (in pancreatectomized-hypophysectomized animals). *b*), The anterior hypophysis greatly increases the severity of pancreatic diabetes. *c*), Resistance to insulin, injected or secreted by a grafted pancreas, precedes and accompanies anterior hypophyseal diabetes. There is, therefore, a functional equilibrium between the anterior hypophysis and the pancreas, which, to a certain extent, act in opposite directions. As the pancreatic tissue is reduced smaller amounts of anterior hypophysis are needed to produce diabetes (53, 55, 61). Reciprocally, when the anterior hypophysis is missing there is hypersensitivity to insulin and the blood sugar and liver glycogen diminish rapidly during fasting.

On the first day of hyperglycemia due to anterior hypophyseal extract the insulin secretion is normal; 2 to 4 days of this hyperglycemia are necessary to produce definite lesions in the pancreatic islets and an appreciable reduction in insulin secretion (60).

The anterior hypophysis is not necessary for the development and maintenance of the pancreatic islets; a physiological pancreatotropic function of the hypophysis acting on the endocrine pancreas cannot be accepted, since hypophysectomy is not followed by a decrease in the weight of the pancreas in dogs (41, 60, 88) and by only a slight one in rats and batrachians (41); in dogs, rats and batrachians the islet mass in-

creases (41, 88) and the insulin content (4) and secretion (41, 60) are normal. If there were any influence of the hypophysis on the islets it would be a slight depressing, not a stimulating one; but there is no definite proof of such an influence.

Anterior hypophyseal extract can produce opposite effects on the islets, either hyperplasia and stimulation or atrophy and hypofunction. The effect obtained depends on the dose of extract, the amount of pancreatic tissue and the species.

The decrease in the capacity of the pancreas to secrete insulin when stimulated by hyperglycemic blood can be demonstrated after 3, 4 or more days of treatment with anterior hypophysis (2 to 3 days of hyperglycemia). A pancreas taken from an animal in this condition and grafted into the circulation of a pancreatectomized dog does not correct the hyperglycemia as does the pancreas from a normal dog (Houssay and Foglia, 59, confirmed by Houssay, Foglia, Smyth, Rietti and Houssay, 60). Campbell et al. (17, 18) and Marks and Young (83) have found that the pancreas from animals with hypophyseal diabetes contains less insulin than the normal pancreas. This decrease can be prevented by early treatment with insulin (17, 29).

Richardson (89, 90) found considerable alterations in the islets; the beta cells lose their granulations, become hydropic and pycnotic and degeneration finally occurs. If the injections are not unduly prolonged regeneration can take place once they are interrupted, but a longer and more intense treatment produces irreversible lesions; the islets are found to have atrophied or to be suffering hyaline degeneration and few beta cells remain (88, 89, 90). An intense treatment produces fatty infiltration, first in the finer glandular ducts, then in acini and finally in the connective tissue; on discontinuing the treatment the fatty infiltration disappears (88).

Anselmino, Herold and Hoffmann (1, 2) reported in 1933 that they had obtained in 3 days a stimulating effect on the pancreatic islets by the injection of anterior hypophyseal extracts. Based on experiments in which only three injections were made this was denied by some observers, but it was later confirmed by them when the injections were continued for 2 to 3 weeks (83). This substance produces hypertrophy of the islets and an increase in insulin content (83); it also produces hypoglycemia if the pancreas is present (1, 2) by increasing the insulin secretion (112).

Dogs that have shown themselves resistant to the anterior hypophyseal extract and have had no hyperglycemia, have normal islets; the cells are not damaged; but sometimes they seem to present a certain degree of hyperfunction (88); the insulin secretion is either normal or increased (60). This is also observed in those dogs which, after having had hyperglycemia, become refractory to the extract and have recovered a normal blood-sugar level (60).

The alterations in the islets are due to the anterior pituitary extract and are not due to the rise in blood sugar, since they do not occur in dogs which have received during 4 days 1 gm. per kg. per day of glucose in a continuous intravenous injection, so as to maintain a blood-sugar level as high as that of the anterior hypophyseal-diabetic dogs. Neither does continuous injection of glucose after interrupting the anterior hypophyseal treatment prevent the cytological recuperation of the islets cells and the normalization of insulin secretion.

Permanent diabetes. When the anterior hypophyseal treatment is discontinued in a dog which has been maintained in a diabetic state for several weeks, if the islets have not suffered too severe damage, they regenerate and the blood sugar returns to normal. In other cases in which the islet lesions are more marked they become irreversible and the animal remains diabetic as long as it lives. This was seen in 1932 in dogs with a reduced pancreas by Houssay, Biasotti and Rietti (53, 55) and demonstrated in a remarkable manner by Young in 1937, in dogs with an intact pancreas. This diabetes

is due to islet deficiency; the exocrine pancreas remains undamaged. This diabetes is not an hypophyseal diabetes and should not be so called; it is really a pancreatic diabetes produced by the harmful action of the anterior pituitary extract on the islets of Langerhans. This permanent diabetes should not be called hypophyseal because the anterior pituitary extract injected has produced the irreversible pancreatic lesions but now is no more acting; it could be named metahypophyseal diabetes, or Young's diabetes, after the man who has studied it in a masterly way. The work of Young (108–111) has been confirmed by Lukens, Dohan, Best, Loubatieres, Houssay and others.

The pancreas of an animal with permanent diabetes does not secrete an appreciable amount of insulin (60); the insulin content is reduced to a mere trace (16 to 18); the islets are atrophied or in hyaline degeneration with few beta cells. The exocrine pancreas does not show any damage. These lesions of the islets explain the permanency of the diabetes.

Young (109–111) has shown that the administration of glucose is followed by a diabetic glycemic curve, the respiratory quotient does not increase and glucose is eliminated in the urine. The insulin need of some of these dogs is greater than in pancreatic diabetes; the sudden interruption of the insulin treatment can produce coma. Nevertheless, it is remarkable that some of these dogs live for a long time (10 months) without insulin. Glycosuria increases when the animals are fed a protein diet, becomes a little less if given a carbohydrate diet, and both glycosuria and ketonuria decrease if a fatty diet is supplied.

Rôle of the adrenals. The adrenals regulate carbohydrate metabolism in a way that resembles to a certain extent the action of the anterior hypophysis (75, 78, 79, 103). Adrenalectomized animals keep a normal carbohydrate level if they are in good condition and are well fed, but fasting rapidly diminishes first the liver glycogen and then the blood sugar. The resynthesis of muscle glycogen after exercise is slower and in advanced insufficiency even the resting muscle glycogen may be reduced. Although adrenalectomy does not prevent the rise in blood sugar in a pancreatectomized dog on discontinuance of the insulin treatment (75), adrenal insufficiency, even treated with salt, attenuates pancreatic diabetes. Long and Lukens (76–79) have shown that in this case survival is prolonged, the increase in blood sugar, glycosuria and ketonuria are less marked and the D:N ratio is low.

This attenuation is not due to the loss of the adrenal medulla since this part of the adrenal can be removed without altering the blood sugar, the liver glycogen or the intensity of pancreatic and phloridzin diabetes, as was shown in 1921 by Houssay and Lewis (63) and has since been repeatedly confirmed (75, 76).

Adrenal extracts and the active steroids of the adrenal cortex increase the liver glycogen markedly and slightly raise the blood sugar of fasting normal, adrenalectomized or hypophysectomized animals. Muscle glycogen is not increased in fasting animals, but does increase when they are fed carbohydrates. These substances improve the resistance to cold and the capacity for prolonged muscular work of adrenalectomized and hypophysectomized animals (see summary by Long et al., 78). Some of the steroids have a diabetogenic activity in pancreatectomized-hypophysectomized animals (78).

Long and Lukens in 1934 and 1936 (76, 79) could not obtain the diabetogenic effect of the anterior hypophyseal extract in adrenalectomized cats. They were careful in stating their conclusions, but this led to the erroneous belief, not shared by Long himself (78), that all the activity of the anterior hypophysis took place through the adrenals. This in spite of the fact that already in 1933 we had shown that the ante-

rior hypophysis produces diabetes in the toad without adrenals, pancreas and hypophysis (47, 49, 62) and in the toad without adrenals and pancreas (62). The diabetogenic effect of the anterior hypophysis can also be demonstrated in dogs with a pancreas reduced to 3 to 4 gm. adrenalectomized and maintained alive with cortical extract (52), desoxycorticosterone or only sodium chloride (unpublished results). On the other hand, 1 mg. of corticosterone or 1 mg. of desoxycorticosterone does not have a diabetogenic effect on the hypophysectomized-pancreatectomized toad, while 1 mg. of anterior hypophysis of toad or mammal has a very pronounced effect.

Up to the present time a typical diabetic condition has not been obtained by the repeated injection of epinephrin or cortico-adrenal hormones, though in high doses these can produce glycosuria (Ingle, 70).

The anterior hypophysis produces a ketogenic effect and increases muscle glycogen in adrenalectomized animals (7, 95, 96). The working capacity of the hypophysectomized rat is less than that of the adrenalectomized (103). Hypophysectomy prolongs hyperglycemia after glucose injection; adrenalectomy does not have this effect, nor does it increase that of hypophysectomy (4, 97).

The action of the anterior hypophysis and that of the adrenal cortex on carbohydrate metabolism are independent of each other, but in some aspects they concur in their effects (78, 95). It is possible that part of the diabetogenic activity of the hypophysis takes place through the adrenals, as Long suggests, but our experiments definitely prove there is a direct action that can be demonstrated in adrenalectomized animals kept in good condition.

Mechanism of the Action of the Anterior Hypophysis

The mechanism by which the anterior hypophysis acts on carbohydrate metabolism is as little known as that of other hormones, insulin for example. Explanations that have been put forward cover only part of the field, and because some are based on results obtained in carnivorous animals, such as the cat and dog, and others on omnivorous or herbivorous animals, such as the rat and rabbit, they are often contradictory.

According to Houssay et al. in dogs (39, 40, 45) and Long et al. in cats (76–79) the anterior hypophysis increases the production of sugar from endogenous protein. Soskin (99, 101) maintains that it also increases the production of sugar from fat. Hypophysectomized dogs can metabolize ingested glucose and protein but the endogenous production of sugar from glucose is diminished (13, 39, 43, 45, 76, 79), and perhaps also that from fat (99, 101). The hypophysectomized dog does not consume more glucose, relatively or absolutely; the basal metabolism is below normal, the non-protein respiratory quotient is normal and increases when glucose is given as in the controls (9, 21, 45). Glucose is utilized, but the glycemic curve is prolonged (4, 9); the amount of glucose oxidized is not above normal (20, 21) and the extrahepatic consumption of sugar is subnormal (99).

The blood sugar and liver glycogen are normal in well-fed hypophysectomized dogs, but both decrease rapidly during fasting (39, 40, 45). Carbohydrate and protein diets, but not a fatty diet, maintain a normal blood sugar and prevent the decrease produced by fasting (13, 38, 45, 101) and phloridzin (11, 48, 54). These animals produce glycogen and glucose normally from ingested protein, but during fasting the endogenous protein catabolism is below normal. This is shown by the low nitrogen and creatinine excretion in a total or protein fast that accompanies the fall in glycogen and blood sugar. In pancreatic and phloridzin diabetes the excretion of glucose and nitrogen and also the blood sugar are lower in the hypophysectomized animals than

in the controls (13, 39, 40, 43, 45, 76, 79); also the production of sugar from fat is lower, according to Soskin (99, 101).

Soskin (101) gives importance to the thyroid in this process and remarks that experiments entailing thyroidectomy are not convincing because of the frequency of accessory thyroid glands. In our experiments thyroid insufficiency was manifest, since the basal metabolism was on average −24 per cent and the fasting nitrogen excretion low. Soskin found that thyroxin prevents hypoglycemia in hypophysectomized dogs and increases the fasting nitrogen excretion, but does not diminish the hypersensitivity to insulin. In our experiments thyroidectomy has very little influence on pancreatic and phloridzin diabetes (11, 13, 39, 40, 45, 48); the effect is considerably less than that due to hypophysectomy.

The hypophysectomized-pancreatectomized dog does not eliminate all of the glucose given (21, 43, 45); part of it is utilized (45, 99), although carbohydrate metabolism is at a very low level (20, 21, 45, 99). The blood sugar falls during fasting, and typical hypoglycemic crises occur which are fatal if glucose is not given in time; the dog is hypersensitive to insulin (43); the isolated tissues can oxidize glucose (98).

Experiments in rats and rabbits have suggested the theory that the anterior hypohysis depresses glucose oxidation. Fisher, Russell and Cori (94) found a relative increase in carbohydrate oxidation in hypophysectomized rats. Greeley (37) observed that hypophysectomized rabbits, both before and after removal of the liver, had a larger consumption of glucose and greater quantities of glucose were needed to keep up their glycemia, than was necessary for normal animals. According to Russell the administration of anterior lobe inhibits the utilization of carbohydrates because these are fixed in the tissues as glycogen (94). The fasting hypophysectomized rat loses more nitrogen than the fasting normal animal (74, 94); this is the opposite of what occurs in the dog.

These differences in certain aspects of carbohydrate metabolism in the cat and dog on one hand, and in the rat and rabbit on the other, are not surprising; many others are known. For example pancreatic diabetes is a very severe disturbance in the cat and dog, while it is less severe in the rat, and even less so in the rabbit, a species in which the tissues have a greater capacity to oxidize glucose in the absence of insulin.

Soskin (99) maintains that the blood-sugar level is regulated by the homeostatic action of the liver which decreases its production of glucose when the blood sugar rises, and increases it when the blood sugar falls. The sensitivity of the liver to changes in blood sugar would be regulated by two opposing factors, insulin and the anterior hypophysis; the thyroid and the adrenals would be accessory factors, governed by the anterior hypophysis and acting in the same direction as this gland. The anterior hypophysis also affects the blood sugar by an extrahepatic mechanism. In the hypophysectomized dog the extrahepatic consumption of glucose is decreased, but the most important factor is the diminished production of glucose by the liver, which keeps the blood-sugar level normal or below normal and facilitates the occurrence of hypoglycemia in the fasting state. Excess of anterior hypophysis acts in the same way as deficiency of insulin, the liver continues to produce glucose in spite of the hyperglycemia, and as the extrahepatic consumption of glucose remains normal, the cause of anterior hypophyseal diabetes would be overproduction of glucose by the liver. When both the anterior hypophysis and the pancreas are removed the liver regulates its sugar production to an intermediate blood-sugar level, lower than in the pancreatectomized but higher than in the hypophysectomized animal. Extrahepatic utilization of sugar in this case would be subnormal. The attenuation of pancreatic

diabetes by hypophysectomy would thus be due to less production of sugar by the liver and not to an increased consumption.

The activity of the anterior hypophysis on ketone bodies is apparently dependent on the liver, which must be functioning for the extract to produce hyperketonemia (Mirsky, 84). Extrahepatic consumption of ketones is not modified in diabetes (Soskins et al., 99).

Young (111) maintains that the injection of anterior hypophysis, when there is enough insulin, increases nitrogen fixation and glucose utilization, producing growth; if the supply of insulin is inadequate, sugar is not utilized but excreted, and a diabetogenic effect is obtained.

There is no proof that hormones form part of the enzyme systems of intermediate carbohydrate metabolism or that they only modify their activity. It is necessary to determine quantitatively their influence on the different chemical reactions and which of these have their velocity or equilibrium modified. Only then will it be possible to understand the action of the anterior hypophysis and other hormones on carbohydrate metabolism.

I must apologize for not having quoted many important papers, several from our own laboratory, in this summary. Reasons of space have made it possible to mention only some of the principal points and only a few of the several thousands of papers that have been published on the subject.

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RESPONSE OF THE VAGO-INSULIN SYSTEM TO ANOXIA AS DEMONSTRATED IN ADRENALECTOMIZED DOGS[1]

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IN THE COURSE of an investigation designed to determine the effects of varying the partial pressures of atmospheric oxygen, nitrogen and carbon dioxide on the occurrence of hypoglycemic convulsions, the authors and their associates (1) discovered the anoxia (induced by breathing a mixture composed of 5% O_2 and 95% N_2) caused a progressive fall in the blood sugar of adrenalectomized dogs without insulin administration. Before operation the same animals showed a typical hyperglycemia from the same degree of asphyxia. Since the latter type of reaction to anoxia in the normal animal is known to be due to excitation of the sympatho-adrenal system with release of adrenalin, as first demonstrated by Cannon and Hoskins (2) and later confirmed by others (3), a well-defined hypoglycemic response (instead of a merely negative one) after adrenalectomy suggested to us that the insulin-producing mechanism (vago-insulin system) might also be stimulated by anoxia with release of insulin.

That this hypoglycemic response to anoxia could not be attributed to depletion of liver glycogen in the adrenalectomized dogs, which showed none of the ordinary evidence of adrenal insufficiency at the time of the experiments, was indicated by the fact that the blood sugar was found to rise spontaneously when the partially asphyxiated animals were again allowed to breathe room air. Furthermore, no effect on blood sugar at all or a slight increase (sympathin effect?) resulted from exposure to an atmosphere composed of 15 per cent CO_2, 20 per cent O_2 and 65 per cent N_2 instead of 5 per cent O_2 plus 95 per cent N_2. The experiments on normal, adrenalectomized and adrenalectomized-depancreatized dogs described here were carried out with the purpose of determining the nature of the physiological mechanism which is concerned with the observed fall in blood sugar, when the latter mixture is inhaled.

While the present studies were still in progress, Feldman, Cortell and Gellhorn (4) confirmed the hypoglycemic effect of induced anoxia in adrenalectomized rats and showed that rats and rabbits with denervated adrenals respond in the same manner as the authors' adrenalectomized dogs did. Metrazol, which, like anoxia, is known to excite the sympatho-adrenal system, caused hyperglycemia in the intact but hypoglycemia in the adrenalectomized rat. Intra-abdominal section of the vagi of the adrenalectomized rats was found to abolish the hypoglycemic response to both anoxia and metrazol administration, further suggesting that the vago-insulin system is stimulated by anoxia but that under normal conditions the effect is masked by the stronger sympatho-adrenal response.

[1] Aided by a grant from the Graduate Medical Research Fund and by the personnel of Work Projects Administration, Official Project No. 165-1-71-124, Sub-project No. 391.

METHODS

Young adult dogs maintained on a mixed stock diet, reasonably complete as regards essentials, were exposed to an atmosphere containing but 4.5 to 5 per cent O_2 to determine their blood sugar response to anoxia. Some of these were then subjected to adrenalectomy while others were pancreatectomized and adrenalectomized. The operations were carried out in two stages, one adrenal (in animals used for studies on adrenalectomy alone), or one adrenal and the pancreas, being removed in the first stage and the second adrenal in the second. Between the first and second operations the depancreatized dogs were given a simple diet consisting of 65 gm. of ground lean beef, 13 gm. of sucrose, 0.2 gm. of NaCl and 0.4 gm. of artificial bone ash per kilogram of body weight daily, together with sufficient insulin to control the diabetes. After total adrenalectomy the basic daily diet contained the following ingredients: casein, 3 gm.; sucrose, 9 gm.; egg yolk, 2 gm.; egg white, 2 gm.; artificial bone ash, 0.4 gm.; and NaCl, 0.5 gm. per kg. of body weight. This was supplemented by 2.5 mg. of ascorbic acid, 5 mg. of nicotinic acid, 2 mg. of thiamine hydrochloride and 3 drops of percomorph liver oil daily. The depancreatized-adrenalectomized dogs were given 100 gm. of ground, raw beef pancreas (or 10 gm. of lecithin) daily in addition. The daily food, cortin and insulin allowances were all divided into two equal parts which were given at 8 A.M. and between 4 and 5 P.M. Water was given *ad libitum* up to the time of each experiment.

After removal of the second adrenal gland, adreno-cortical extract[2] was given subcutaneously twice daily in doses varying between 10 and 5 cc. to prevent adrenal insufficiency until the operative wounds had entirely healed. Thereafter from 2 to 4 cc. per day sufficed to prevent the usual manifestations of insufficiency. In the depancreatized animals, the insulin requirement fell from between 8 and 15 U per day before the second adrenal was removed to between 2 and 4 U per day thereafter. Food cortin and insulin were all withheld for 15 hours before every acute experiment. Throughout each control and each experimental period, the animal was kept loosely fastened to a board in an ordinary clinical oxygen tent maintained at atmospheric pressure. In preparation for the experiment pure N_2 was mixed with the circulating room air in the tent in sufficient amounts to reduce the O_2 content of the latter to the desired partial pressure (approximately 38 mm. Hg). After the tent was adjusted, the O_2 and CO_2 contents were determined by the Orsat method at 20-minute intervals throughout the entire period of observation. The CO_2 content was kept below 0.5 per cent by absorption with soda lime. The tendency of animals to struggle during experiments involving reduction of the partial pressure of O_2 was minimized by training and by making the change from room air to the low-O_2 mixture gradually. Blood samples were taken for sugar, inorganic phosphate and potassium determinations at the beginning of each experiment and at hourly or half-hourly intervals thereafter. The potassium and inorganic phosphate were also determined in some of the experiments. Methods of analysis were those used in previous investigations (5).

RESULTS

Representative data are presented in the accompanying graphic charts which are largely self-explanatory. Those shown in figure 1 illustrate the difference between the blood glucose responses of normal dogs and those of adrenalectomized dogs subjected to anoxic anoxia. The blood sugar curves shown in the first panel represent increases of between 30 and 150 per cent above fasting blood glucose levels during a period of

[2] The adrenal cortical extract was supplied by the Upjohn Co., Kalamazoo, Mich.

2.5 hours, when normal dogs were made to breathe a gaseous mixture containing 4.5 to 5 per cent O_2. Those in the panel on the right from adrenalectomized animals subjected to anoxia represent decreases in blood glucose values varying between 15 and 60 per cent below the fasting levels. In the latter animals the blood sugar was found to rise again after room air was admitted to the tent.

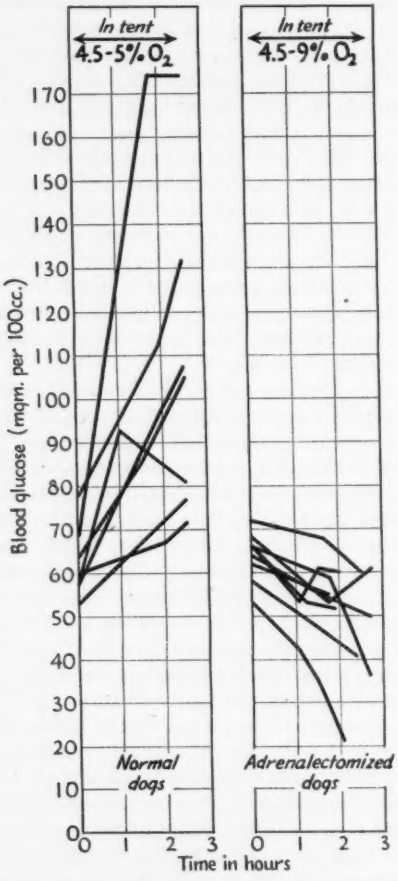

Fig. 1. Effects of anoxia on the blood glucose of normal fasting dogs (panel on left) and on that of adrenalectomized dogs (panel on right) under the same conditions.

The inorganic phosphate and potassium of the plasma were also determined in a number of the foregoing experiments. Both of these constituents were found to fall below their original levels as a result of the anoxia, whether the blood sugar was rising, as in the normal dogs, or falling, as in the adrenalectomized animals. The decreases were of the same order of magnitude in the normal as in the adrenalectomized animals. In both instances this response was tentatively interpreted as an indication of insulin release into the blood stream (6). Samples of data pertaining to this phenomenon are presented in figure 2, which is self-explanatory. Any transient elevation in the plasma

K which may have occurred in the normal animals at the beginning of the period of anoxia, corresponding with that observed by D'Silva (7) and others (8) after administration of adrenalin, would not be evident in the samples taken one to one and one-half hours later.

Two dogs, which were totally depancreatized and adrenalectomized, were subjected to anoxia for periods of 2.5 hours at approximately 10-day intervals. Blood sugar

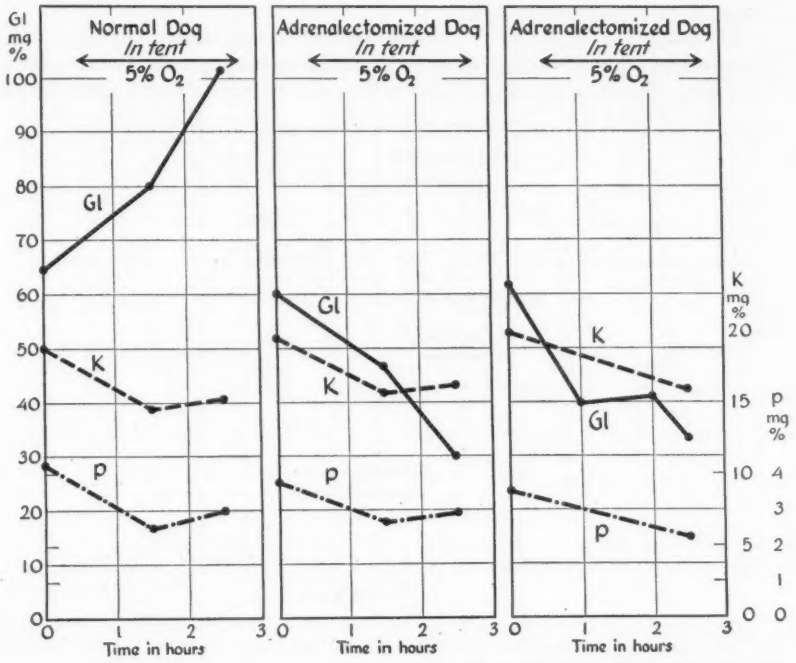

Fig. 2. Effects of anoxia on plasma potassium (K) and inorganic phosphate (P) as well as on blood glucose (gl.) in normal and in adrenalectomized dogs.

determinations were made every hour or half-hour throughout each such experimental period. During the intervals between regular experiments, control blood glucose curves were obtained on the same animals while they breathed room air. The data obtained from this phase of the study are presented graphically in figure 3. It is apparent from the blood sugar curves shown in the panel on the right that the hyperglycemic response to anoxia characteristic of the normal animal was extremely feeble, if not entirely lacking, in these dogs and that the hypoglycemic response observed after simple adrenalectomy was totally absent, excepting in one experiment on each animal carried out on the same day (curves shown by broken lines). The only respect in which the conditions of the experiments producing these two hypoglycemic curves are known to have differed from all of the others was that protamine insulin instead of regular insulin was given by mistake on the evening before the experiments. A satisfactory explanation for the intensification of protamine insulin action by anoxia, if such it be, is not available at the present time. The very slight upward tendency of several of the other curves in this panel might possibly be attributed to release

of sympathin E into the blood stream as a result of anoxia. Examination of the control blood sugar curves presented in the left panel of the figure shows that small variations in the directions taken by the curves occurred even when room air was respired. Plasma potassium and inorganic phosphorus were not determined along with blood sugar in most of the experiments on the adrenalectomized-depancreatized dogs. However, from the few measurements that were made, it was apparent that the decreases observed in normal and in adrenalectomized animals did not occur. In a few instances the potassium rose slightly.

In previous investigations (1, 5) it was observed that the blood sugar curve of the normal dog in a state of insulin hypoglycemia always showed a prompt rise in response to anoxia, whereas in the adrenalectomized animal the hypoglycemic action of the

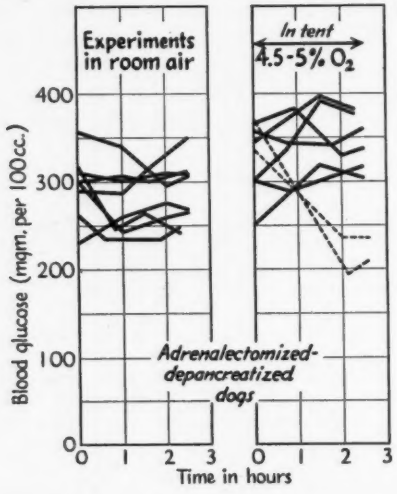

Fig. 3. Effects of anoxia on blood glucose curves of adrenalectomized depancreatized dogs (panel on right). Compare curves with those obtained on the same animals when the latter breathed room air instead of 5% O_2 (panel on left).

insulin appeared to be accentuated by this factor. Experiments were, therefore, carried out on two adrenalectomized-depancreatized dogs to determine whether or not either of these responses to anoxia could be elicited. The essential data are presented graphically in figure 4. The left hand panel in the figure shows the effect of anoxia on the descending blood sugar curves of two normal animals which had received massive doses of insulin several hours previously. The rise in blood sugar was fairly striking but was not sustained throughout the period of anoxia in either case. The hypoglycemic curves (due to insulin) of the adrenalectomized-depancreatized dogs, shown in the panel on the right, were obviously not altered materially in either direction by anoxia.

COMMENT

The experiments briefly reported here furnish direct evidence in support of the interpretation offered tentatively in the authors' previous communication (1), concerning the hypoglycemic response to anoxia on the part of adrenalectomized dogs, namely, that the latter is due to excitation of the insulin-producing mechanism (vago-insulin system) by oxygen lack. Whereas the normal fasting dog (maintained on a

mixed diet up to 14 hours before the experiment) regularly shows a rise in blood sugar when made to breathe an atmosphere containing but 5 per cent oxygen, the adrenalectomized animal shows a significant fall under the same conditions. The potassium and inorganic phosphate of the plasma are decreased in the adrenalectomized as well as in the normal animal. Following complete removal of the pancreas as well as the adrenals, no comparable changes in these constituents or in the blood sugar are found to occur as a result of anoxia. While in the normal dog the downward trend of the blood sugar curve, following insulin administration, is interrupted by a sharp rise,

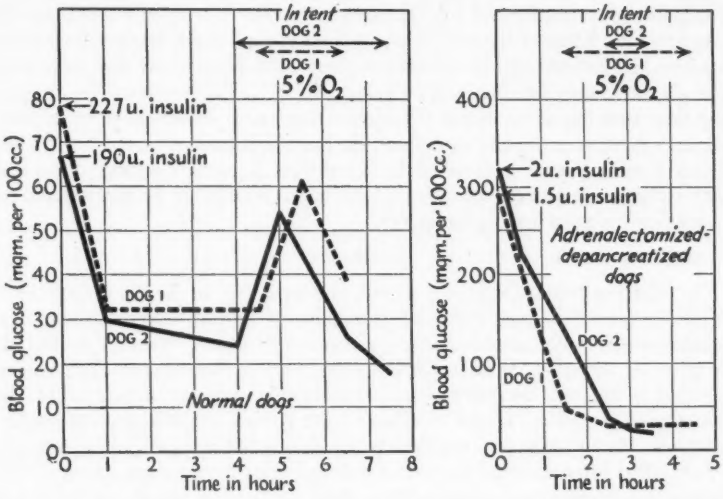

Fig. 4. Effects of anoxia on blood glucose during periods of insulin hypoglycemia. Note rise in curves of normal dogs and the absence of any effect in those of the pancreatectomized-adrenalectomized animals.

when the oxygen content of the atmosphere is suddenly reduced, there is either no alteration at all or a slight acceleration in the rate of fall in the adrenalectomized animal. The pancreatectomized-adrenalectomized dog shows neither of these effects on the hypoglycemic curve.

Taken together, the foregoing facts indicate that both the sympatho-adrenal and the vago-insulin systems are stimulated by anoxia. The stronger response of the former masks that of the latter, however, under normal conditions, as already pointed out. Emotional disturbances and sham rage, earlier shown by Cannon (9) to stimulate the sympatho-adrenal system in the normal animal with production of hyperglycemia, were recently shown by Gellhorn and his collaborators (10) to elicit a hypoglycemic response in rats after denervation of the adrenals or after section of the spinal cord. Adreno-demedullated-vagotomized rats did not show this response. Certain psychotic patients were found by these workers to develop an increased insulin content of the blood following emotional excitement. This was sufficient in amount in some cases to offset the hyperglycemic response to adrenal stimulation.

That certain species of animals and some human subjects may exhibit a dominance of the vago-insulin over the sympatho-adrenal system, as regards blood sugar response to anoxia or emotional stimuli, is not inconceivable. The serious hypoglycemic reactions which occur in the snowshoe hare (*Lepus americans*) under conditions of stress

or fright may well be an example among lower animal species. This interesting phenomenon, first described by Green and his coworkers (11) is thought by them to be an essential feature of *shock disease*, a disorder which results in widespread devastation among these animals in their wild state. In captivity they succumb within a few days. When frightened, animals apparently in full health suddenly develop typical signs of profound hypoglycemia and expire unless glucose is administered promptly.

In spite of extensive investigations on this aspect of the problem, no infectious or toxic agent has as yet been shown to be of etiological importance. Postmortem examination reveals no morphological changes in the pancreas. Many but not all of the animals show atrophic and fatty changes in the liver. Glycogen stores in the liver are usually low. Most of the rabbits dying from typical shock disease show marked congestion and hemorrhages in the adrenal glands. It is conceivable that these lesions in the adrenals might interfere with glycogenesis in the liver (cortical function) and at the same time impair or abolish the adrenal response to emotional or other stimuli (medullary function), thereby disturbing the balance between the sympatho-adrenal and vago-insulin systems in favor of the latter. Certain cases of hypoglycemia in the human subject, not otherwise accounted for, might reasonably be investigated from the viewpoint of vago-insulin dominance.

SUMMARY

The effects of anoxia (breathing 5% O_2 plus 95% N_2) on the blood sugar and on the plasma potassium and inorganic phosphate of normal, adrenalectomized and adrenalectomized-pancreatectomized dogs were determined. Whereas normal dogs showed a rise, adrenalectomized animals showed a fall in blood sugar. The changes in sugar level in both of these states were accompanied by decreases in plasma potassium and inorganic phosphate, similar to those resulting from administration of insulin in small doses. The hypoglycemic response to anoxia was not obtained when pancreatectomy as well as adrenalectomy was performed, excepting in two experiments in which protamine insulin was inadvertently given instead of regular insulin on the day preceding the experiments. No significant decrease in the potassium or the inorganic phosphate of the plasma was produced by anoxia in the pancreatectomized-adrenalectomized dogs. A slight elevation of the blood sugar in response to anoxia in some of the experiments on these animals suggested release of sympathin E into the blood stream.

These results are interpreted as evidence that the vago-insulin, as well as the sympatho-adrenal, system is excited by asphyxia. Although the response of the latter overshadows that of the former in most normal animals, the possibility of a reversal of dominance under certain conditions is discussed.

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PREVENTION OF EXPERIMENTAL GASTROJEJUNAL ULCER BY ENTEROGASTRONE THERAPY[1]

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IT HAS BEEN CLEARLY ESTABLISHED that the parenteral administration of an extract of the upper intestinal mucosa, containing a chalone called enterogastrone, depresses the motor and secretory activity of the stomach (1). In view of the anticipated potentialities of such an agent in the treatment of peptic ulcer, a therapeutic trial has been made in a series of dogs prepared by the Mann-Williamson (M-W) operation.

The Mann-Williamson operation consists of a gastrojejunostomy with drainage of the pancreatic juice and bile into the distal ileum (last 20–25 cm.). This type of preparation for the production of peptic ulcer was chosen for several reasons. First, a jejunal ulcer occurs uniformly in 'all' dogs (98.2% of 114) unless some form of therapy is used. Second, it is analogous to the jejunal ulcer which not infrequently occurs after a gastrojejunostomy in man and which is so difficult to manage therapeutically. Third, it has been rather clearly demonstrated that the ulcer is chiefly due to the corrosion of the jejunal mucosa by gastric juice, to which the jejunal mucosa is more sensitive than the duodenal mucosa. A nutritional disturbance resulting from incomplete intestinal digestion is a factor which facilitates the onset of the ulcer and the consequent lethal perforation or hemorrhage. Fourth, it has been shown that most M-W dogs manifest a hypercontinuous secretion (2), and that procedures designed to reduce the secretion of gastric juice, such as fundusectomy (3) and the oral administration of aluminum phosphate gel (4) are potent in preventing this experimental ulcer.

METHODS

Diet. All animals, control and treated, received a diet consisting approximately of 200 gm. of raw ground pancreas, 200 gm. of raw ground liver, 100 cc. of milk, 450 gm. of a commercial dog food (Pard) and 8 cc. of fish liver oil. The diet was fed twice daily *ad libitum*. To prevent a secondary anemia which some M-W dogs manifest even before they develop an ulcer, 1 gm. of ferrous sulphate was mixed with the diet daily.

Untreated control animals. Sixteen control M-W animals receiving this diet in an investigation just preceding this one (4) died with ulcer in from 2 to 5 months, the average survival time being 17 weeks. All of 42 dogs receiving our stock diet, or the same diet without the raw pancreas and liver, died of ulcer in 17 weeks, the average survival time being 11 weeks (5). Thus, it is clear that the raw pancreas and liver has some prophylactic value.

In table 1 we present a summary of the longevity data for 114 M-W dogs which

[1] Aided by a grant from the Josiah Macy, Jr. Foundation.

we have observed during the last 5 years and which received the special diet without benefit of any other therapy. It is to be noted that by the end of 9 months close to 97 per cent were dead with ulcer.

Muscle extract-treated control animals. Ten dogs were treated with an extract of pork muscle prepared according to the method for making the enterogastrone preparation (*vide infra*). The muscle extract was administered to the dogs in the same amount and at the same frequency as the enterogastrone preparation. It was assayed and found to manifest no inhibition of gastric secretion or motility. This type of control experiment was considered to be essential to determine the specificity of the enterogastrone preparation used.

Enterogastrone-treated animals. Twenty-five animals were treated with our preparation of enterogastrone. They were injected intravenously at about 10 A.M.,

TABLE 1. LONGEVITY OF 112 MANN-WILLIAMSON DOGS DYING WITH ULCER WITHOUT BENEFIT OF THERAPY, EXCEPT FOR A DIET CONTAINING RAW PANCREAS AND LIVER AND SOME MILK TWICE DAILY *ad libitum*

Mean longevity of the 112 dogs, 15.2 weeks[1]

	Weeks								
	4–7	8–11	12–15	16–19	20–23	24–27	28–31	32–35	36–39
Number dying during each period	8	34	31	15	8	6	5	3	2
Per cent dying during each period	7.1	30.3	27.7	13.4	7.1	5.4	4.4	2.7	1.8
Per cent dead by end of each period	7.1	37.4	65.1	78.5	85.6	91.0	95.4	98.1	99.9

[1] This average and the data in the table do not include 2 animals which survived for more than 90 weeks; thus, 2 (or 1.8% of 114 animals) did not develop ulcer in almost two years.

4 P.M. and 10:30 P.M. with 50 mg. of the preparation dissolved in a small quantity of water every day, except Sunday, when the animals received only two injections.

A dose of 50 mg. of the preparation, which was assayed intermittently to insure the uniformity of the product, contained approximately one 'unit' of enterogastrone. That is, it halved the gastric secretory response of a total-pouch dog using the double-histamine method of assay (5).

The procedure for preparation of the enterogastrone used on the M-W dogs was essentially that of Gray, Bradley and Ivy (1). A smooth suspension was made of 1 kg. of 'A' precipitate (7) in 5 liters of tap water, and the pH of this suspension was adjusted to 5.4 by the addition of strong sodium hydroxide solution with constant stirring. The pH measurements were made with a Coleman glass electrode apparatus. This adjusted mixture was heated to boiling by a current of live steam and boiled for 2 to 3 minutes, and then filtered hot through a filter paper of good quality. The filtrate was cooled with running water and treated with a saturated solution of tannic acid to the point of complete precipitation; and the resulting flocculent precipitate was collected by centrifugation, washed with distilled water, and recentrifuged until packed hard in the cups.

The tannate was decomposed by thorough trituration with slightly more than its own volume of 70 per cent aqueous acetone containing 2 per cent of concentrated hydrochloric acid, centrifugation, and filtration of the supernatant liquid through a retention filter paper (Whatman 40 or 42). The insoluble, inert residue was washed

with the acid-acetone mixture and the washings combined with the original filtrates, which was then treated with 8 to 10 volumes of pure acetone. The resulting white amorphous precipitate was centrifuged out, washed with acetone, acetone-ether mixture, and finally ether, and air-dried and powdered.

The muscle extract was prepared from the filtrate of a suspension of lean pork meat in 0.4 per cent HCl which was saturated with salt, and the resulting precipitate was treated exactly like the 'A' precipitate except that the pH was adjusted to 8.2, the isoelectric point of the particular material.

Other Methods. Temperature reactions. On several occasions the body temperature of the animals was recorded hourly for 5 hours after the injection of the extracts. The injections caused no significant changes in body temperature. *Examination of the blood.* Every two months a determination of erythrocyte and leucocyte count, hemoglobin and red cell hematocrit was made. *Gastric analyses* were made at intervals throughout the course of the experiment. The regular meal was fed and then a sample of gastric contents was removed at the 3rd, 4th and 5th hours. This was done in the control and treated animals and with and without the injection of the muscle extract or of enterogastrone. *Liver function test.* The Rose Bengal test (8) was applied to some of the dogs, the method being slightly modified by us in regard to dose and the use of a photelometer. This test was chosen because it has been found in our laboratory (Dr. S. Freeman) that it is the most sensitive test in revealing relatively minor hepatic damage; i.e., the test becomes abnormal about 3 weeks after a dog is placed on a low protein diet.

RESULTS

Muscle extract-treated or control group. The essential observations are recorded in table 2. It is to be noted that all of the dogs died with ulcer in from 5 to 30 weeks, the average survival time being about 16 weeks. This average survival time is ap-

TABLE 2. CONTROL ANIMALS TREATED WITH MUSCLE EXTRACT ALL OF WHICH DEVELOPED ULCER

Dog Number	Duration in Weeks	Ulcer	Loss in Weight, %	Remarks
1C	5	Large, 3.5×1.5 cm., indurated	33	Marked loss of weight; diagnosis of ulcer made week prior to death
2C	30	Chronic indurated perforated jejunal 4×1.5 cm.	12	Diagnosis of ulcer made 2 months prior to death; marked polyphagia
3C	15	Chronic indurated jejunal	35	Diagnosis of ulcer made 2 months prior to death
4C	16	Chronic perforated jejunal	40	Diagnosis of ulcer made 1 week prior to death
5C	25	Large jejunal	50	Diagnosis of ulcer made 6 weeks prior to death
6C	12	Chronic indurated 2.5×2 cm.	25	Diagnosis of ulcer made 6 weeks prior to death
7C	4	Small jejunal ulcer		Pneumonia
8C	27	Ulcer present chronic, indurated	40	Diagnosis of ulcer made Aug. 13, 1941; dog died Dec. 19, 1941; ulcer present for 4 months
9C	16	Chronic indurated jejunum 2.5×2 cm. perforated	8	Diagnosis of ulcer made 1 month prior to death
10C	7	Chronic perforated jejunum, 2.5 cm. diameter	30	Dog developed marked jaundice
Average	15.7			

TABLE 3. ENTEROGASTRONE TREATED DOGS

Dog Number	Date Operated	Ulcer	Change in Weight, %		Longevity in Weeks[1]	Notes
			Loss	Gain		
11 Dogs still alive without ulcer for more than 9 months						
3	9/25/40	None	0	+	73	Explored at 1 year; stopped therapy; no ulcer to date
4	10/ 1/40	None	0	+	72	Same
5	10/14/40	None	0	+	70	Same
6	10/18/40	None	0	+	69	Same
7	10/22/40	None	0	+	69	Same
10	11/22/40	None	0	+	65	Same
13	1/ 2/41	None	0	+	60	Same
16	3/29/41	None	0	+	48	Will be explored at 1 year
18	5/26/41	None	0	+	37	Will be explored at 1 year
21	4/16/41	None	0	+	46	Will be explored at 1 year
25	6/ 1/41	None	0	+	36	Will be explored at 1 year
8 Dogs dying within 9 months without ulcer						
2	9/23/40	None	10	0	20	Death due to pneumonia
8	10/24/40	None	0	0	7	Death due to pneumonia
12	12/30/40	None	0	+	33	Death unexplained
14	1/15/41	None	25	0	29	Adhesions and obstruction
15	3/ 1/41	None	0	+	36	Death unexplained
19	4/21/41	None	50	0	15	Adhesions and obstruction
20	4/14/41	None	40	0	7	Adhesions and obstruction
22	4/29/41	None	20	0	6	Adhesions and obstruction
Average					19	
6 Dogs dying within 1 year with ulcer						
1	9/20/40	2	40	0	37	Perforation
9	10/21/40	1	0	20	17	Perforation
11	11/25/40	1	20	0	19	Chronic hemorrhage
17	4/ 2/41	1	40	0	25	Perforation
23	5/ 9/41	3	20	0	32	Perforation
24	5/14/41	2	15	0	32	Perforation
Average					27	

[1] As of March 1, 1942.

proximately the same as that of the 16 control dogs (17 weeks) which were maintained on the same diet and which did not receive injections of muscle extract. It also falls in line with the longevity of 112 animals receiving the same diet (see table 1).

On the basis of these results, the injection of the muscle extract had no value in preventing or delaying the development of ulcer.

Enterogastrone-treated group. The essential observations are recorded in table 3. It is to be noted that only 6 of the 25 animals treated with enterogastrone, or 24 per cent, developed ulcer. The average survival time of the 6 that did develop ulcer was 27 weeks or almost twice that of the control group.

The remarkable observation is that 11 animals have survived more than 9 months without developing ulcer and 7 of the 11 well over a year.

At one year 7 of the 11 animals were explored, the jejunal mucosa visualized, and no ulcer was found. The enterogastrone therapy was discontinued. It is striking that 4 of the 7 animals have now received no therapy for

over 4 months and none have developed ulcer. This is remarkable because in a previous study (4) when 10 M-W dogs, in which ulcer had been prevented for from 9 to 11 months by aluminum phosphate gel, were deprived of therapy an ulcer developed in from 5 to 16 weeks.

In table 3 it is to be noted that 9 of the enterogastrone-treated animals died within 9 months without an ulcer. That this may occur when some sort of effective therapy is administered has been noted in our previous investigations (2–5). This simply means that the operation *per se* is attended by a mortality because adhesions and intra-abdominal hernia occur and cause death from intestinal obstruction or predispose to distemper and pneumonia.

Anemia. The erythrocyte and hemoglobin determinations showed that anemia did not occur as the result of therapy, since anemia only occurred when an ulcer developed; then, of course, it was due to hemorrhage and the presence of blood was detected in the stools.

Rose Bengal test of liver function. The Rose Bengal dye test was applied to dogs 4, 5, 6, 7, 10, 13, 16, 18, 21, 24, and 25. Normal values were obtained in all dogs except 24: which had an ulcer. That tests of liver function may yield low values in M-W dogs a short time before death from perforation or hemorrhage has been observed before (9). We performed the test chiefly for the purpose of attempting to

TABLE 4. AVERAGED RESULTS OF FREE AND TOTAL ACIDITY OF GASTRIC CONTENTS IN MUSCLE-EXTRACT CONTROL AND ENTEROGASTRONE-TREATED ANIMALS

	3rd Hour Acidity[1]		4th Hour Acidity[1]		5th Hour Acidity[1]	
	Free	Total	Free	Total	Free	Total
Controls; treated with muscle extract	5	81	5	87	5	108
Enterogastrone-treated group	5	85	5	85	6	85

[1] Clinical units of acidity.

ascertain whether the repeated enterogastrone injections might affect the liver. The postmortem examination of the livers of those dogs surviving less than 9 months revealed no injury from enterogastrone. However, it will be necessary to delay the answer to this question until the livers of the animals receiving enterogastrone for one year become available for histologic study. The results to date show only that the 7 dogs receiving enterogastrone for one year manifested no evidence of disturbance of liver function as measured by the Rose Bengal test (8).

Gastric analysis. The averaged results of the titration of the free and total acidity of the aspirated samples at the 3rd, 4th and 5th hours are shown in table 4. The total acidity of the muscle-extract controls at the 5th hour is definitely greater than that of the enterogastrone-treated animals. This trend was anticipated because it has been found that most M-W dogs manifest a hypercontinuous secretion (2, 3, 5). However, the difference was not as striking as we had anticipated.

It was realized at the time that a better method of ascertaining the effect of the treatment on gastric secretion in the two groups of animals should be employed. This was not feasible because of lack of time (the making of the extracts and the injections consumed much time) and also because we did not desire to intubate these animals excessively since we were primarily interested in therapeutic results.

DISCUSSION

The therapeutic results require little discussion. They show that the enterogastrone preparation possessed a marked potency in preventing the development of

ulcer in Mann-Williamson dogs. Why ulcer occurred in 6 of the 24 dogs requires some consideration. These factors are worthy of mention: *a*). Perhaps one should not expect to prevent ulcer in all M-W dogs simply by making three injections of enterogastrone daily when it is realized that the depression of gastric secretion from one injection does not endure longer than two hours. *b*). Another, perhaps more pertinent, reason is that our preparation is impure and some dogs become refractory (10).

It has been reported that 2 of 8 total-pouch dogs (25%) used for assay of enterogastrone preparations became refractory within one month, whereas the remaining 6 gave no indications of altered tolerance on receiving repeated injections for periods varying from several months to several years. In one of the refractory animals it was possible to show that the refractoriness was directed towards the extract and not to the chalone, since the secretion of the animal's stomach was inhibited when fat was fed (10). It is a striking coincidence that 25 per cent of total-pouch dogs became refractory to the extract and that 24 per cent of our M-W dogs developed ulcer. It was not practically feasible to test the M-W dogs to determine whether they had become refractory.

The results on the control group of animals treated with muscle extract show that the enterogastrone extract had a specific effect. This specific effect on the basis of existing knowledge is attributable to the depression of gastric secretion caused by enterogastrone. That this interpretation may be inadequate is suggested by the fact that 5 animals have not developed ulcer although they have received no therapy for 4 months or longer. On the basis for previous experience when therapy has been withdrawn ulcer has developed within 4 months (4). This indicates that the repeated injection of enterogastrone produced some long-standing effect. Whether this long-standing effect is due to the chalone or to some special 'impurity' in the extract remains to be determined. It is possible that the enterogastrone extract is exerting some effect other than the depression of gastric secretion and motility, such as an epithelial growth-promoting influence. Repeated injections (3 times daily) of enterogastrone may lead to a permanent decrease in the gastric secretory response to a meal, and in time even to histamine. The effect of feeding a high fat diet three times daily for a prolonged period is unknown. In the experiments of Gray and Wieczorowski (10) in which no change in response to histamine was observed in 6 of 8 dogs, the enterogastrone was injected only once each day 2 or 3 times per week. Also, some 'impurity' in the enterogastrone extract, and not present in the muscle extract, when injected repeatedly may have had a long-standing depressing effect on the gastric secretory cells (11). These are questions which require study by specially designed experimental procedures; such experiments are now under way. It cannot be concluded that the ulcer-preventative effect is due to enterogastrone until the effective principal has been chemically isolated and identified.

SUMMARY AND CONCLUSIONS

Thirty-five M-W dogs were prepared and fed twice daily *ad libitum* a special diet. The average longevity of 112 such animals, on this diet, dying with jejunal ulcer was 15.2 weeks. Only 1.7 per cent of 114 animals did not develop ulcer. About 97 per cent of 114 animals had died of ulcer within 9 months. Seventy-eight per cent had died of ulcer within 4 months.

Ten of the 35 M-W dogs were injected intravenously 3 times daily with an extract of pork muscle prepared by the method used for making an enterogastrone extract of the upper intestinal mucosa of the hog. The muscle extract did not inhibit

gastric secretion and motility. All of the 10 died of jejunal ulcer in from 4 to 30 weeks, the average survival being 15.7 weeks.

Twenty-five of the 35 M-W dogs were injected intravenously with a preparation containing enterogastrone. Only 6 of the 25 dogs, or 24 per cent, developed jejunal ulcer. Eight of the 25 died within 9 months without ulcer; they lived an average of 19 weeks without developing ulcer. Eleven are still alive without ulcer for more than 9 months, 7 of the 11 more than one year. Five of these latter 7 have been without treatment for from 4 to 5 months without developing ulcer.

These data show that the enterogastrone extract employed, even though impure and known to produce refractoriness in about 25 per cent of dogs, has a remarkable potency in preventing jejunal ulcer in M-W dogs, and indicate that the extract on repeated administration produces rather long-standing 'immunity' against the development of jejunal ulcer.

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COMMERCIAL ENDOCRINE PREPARATIONS

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THE DEVELOPMENT of both experimental and clinical endocrinology is bound up necessarily with those sources which can furnish large amounts of animal gland tissue for use in replacement therapy. Thus contact with commercial abbatoirs, and eventually with firms interested in manufacture of processed gland extracts has been a feature of this field of medical research. It would be manifestly unfair to expect that such business organizations should remain merely the donors for the supply of hormone-containing extracts, without any opportunity to derive revenue from sale of these materials when their use has been adopted widely. Nevertheless there is frequently a tendency for the investigator to speak disparagingly of the commercial firm and its products, as compared with the materials prepared in the laboratory of the academic investigator. The differences are often merely a result of the lag, measured in terms of only a few years, between the newest discovery and the current practice of the general practitioner in medicine. The common prescriptions by physicians and the volume production by the pharmaceutical manufacturers are usually based on what were considered advanced research a few years before. Illustrating this lag it may be noted that in 1940 the United States Government was engaged in a legal attempt to stop production and sale of a certain type of extract from the ovaries of animals, as being archaic and useless. This very material had been introduced by the defendant firm in 1912 in response to discoveries and requests emanating from research workers in this country, who believed that the 'new' ovarian extract represented a distinct advance in securing potent hormone material. Since the lifetime of one investigator or physician can so readily cover the change from new discovery to obsolete and relatively useless material, the problem of evaluating commercial endocrine extracts becomes a perennial task. Such an appraisal, from the viewpoint of a clinical investigator, is offered as a contribution to the desirable agreement on therapeutic endocrine preparations which may help in both clarity of thinking and economy of gland tissues.

THYROID

The standard preparation of thyroid has been for many years the desiccated gland substance, powdered, and diluted with inert material for tablet manufacture, according to conditions prescribed by the U. S. Pharmacopoeia. The potency of this dried thyroid is specified in terms of its content of iodine, 0.2 per cent. Attempts have been made to demonstrate other measures of potency, based on direct biological tests, but to date none of these is accepted. This dried gland material is reasonably stable, so that one may order with confidence a supply of tablets for several months at one time. Far more important than the freshness of the preparation, formerly urged, is the care with which the thyroid tablets have been standardized to uniformity in iodine content. Although this seems such a simple and elementary point, the clinical errors

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based on ignoring this matter continue to be widespread. Difficulty derives from the fact that there exist in the United States three other competing types of thyroid tablets: *a*), some standardized acccording to the British Pharmacopoeia, at a much lower iodine content; *b*), others at 0.3 per cent iodine and therefore presumably half again as potent as the U.S.P. type; and *c*), a variety of tablets which are not of any known or labeled potency. It will be apparent that any one of the first three types mentioned may be used with dependability and with equally uniform clinical activity when changing from one lot to a successive purchase of the same variety. But this cannot be said of unstandardized thyroid tablets, which ought never to be employed by any clinician. Furthermore, it is highly important that whenever a physician writes his prescriptions for dried thyroid, he should specify whichever type he prefers to use. In refilling prescriptions attention to this point is equally essential. Ignorance of these matters often explains failures to continue the benefits of use of thyroid, or the sudden appearance of toxic symptoms.

Many attempts have been made to prepare extracts from the thyroid which would have greater potency, or possess advantages over the whole dried gland. Although some of these extracts are active, clinically useful and stable enough for commercial production, none of them has withstood the test of extensive clinical use. This means merely that they possess no demonstrable advantage over the simple dried tissue. In recent years it has been claimed that a separation can be made between hormone materials which cause increase in metabolism and those which induce tachycardia. Such advantages have not been given wide credence. There is no such recognized material in American pharmacy as 'thyroid extract.'

The preparation of thyroxine, a pure substance which can be made synthetically or which may be derived from chemical extractions of the thyroid, and which will do the same things physiologically as the whole thyroid, seemed to be the answer to the desire for the pure hormone of the gland. There are at least two difficulties besides the greater cost of the chemically pure substance. The first is that thyroxine is so poorly soluble in water that it must be dissolved with the aid of very exact amounts of alkali, and that for most dependable use it must be injected intravenously. The second is that there is good evidence for doubting that thyroxine is the actual form in which the hormone circulates and acts in the body, *i.e.* it is probably a part of some larger molecular structure, as yet unknown. Because of these difficulties thyroxine is now regarded as a chemical of much interest for research, but of no present importance in clinical practice.

Advantage has also been claimed for specially coated tablets of thyroid, due to suspected destruction of the thyroid substance in the upper portion of the digestive tract. There is no adequate evidence for this assumption. It is probably of no value to delay the absorption of thyroid, or to coat it to avoid action of digestive enzymes. The special coatings may have two disadvantages: increasing the cost of tablets without added clinical value, and delaying absorption so long that the tablets pass out with the stool at times, without clinical benefit. It is therefore advised that specially coated tablets of thyroid be shunned.

Thyroid tablets vary from 0.1 to 2 grains. It is doubtful that any tablet smaller than 0.25 grain is required in even pediatric practice, since completely cretin or myxedemetous patients require about 2 to 4 grains daily. It is questionable if one can detect deficiencies of an order that require less than one-tenth the maintenance dose for these thyroidless subjects. Since the absorption of the hormones goes on slowly, and since the physiological results do not appear for many hours after a tablet is ingested, there is no adequate reason for using thyroid in divided doses at two or more

times during the day. It is better clinical practice to give the daily requirement at one regular daily dose. Far more important is the understanding by physician and patient that the long-continued activity of thyroid leads to cumulative drug action, so that maximal benefits (or maximal toxicity from overdoses) appear only after two months or longer from the initiation of a given daily dosage.

PARATHYROID

Clinical use of parathyroid gland preparations contrasts in many ways to that of thyroid. The gland substance is not dependably active if given orally, and it is therefore no longer considered good practice to prescribe parathyroid tablets. Consequently extracts must be prepared for injection. This became possible as a consequence of discoveries by Collip and Hanson, and for fifteen years there have been available three commercial preparations of such parathyroid gland extracts. These are now standardized in terms of the same unit, based on biological assay depending on the ability of the extract to elevate the serum calcium level in dogs. Unfortunately these extracts are still essentially crude, containing much protein which is probably inert from an endocrine point of view, but they have served a useful purpose in treatment of tetany due to parathyroid deficiency. In some clinical cases the continued use of these preparations has led to a state of resistance which requires greatly increased dosage. No explanation for this resistant state has been furnished, and the amount of the material used has been so small that no manufacturing firm has seen fit to apply to the problem of purification of the extract the professional attention probably needed to secure a refined hormone product. At about the same time that the factors were being discovered it was shown that clinical control of parathyroid tetany could be maintained with ease by use of either vitamin D or a special irradiation product of ergosterol called dihydrotachysterol. Although there is still room for differences of opinion about the completeness with which these vitamin compounds replace the action of the gland, clinicians seldom use the parathyroid extracts save for emergencies of acute tetanic seizures.

PANCREAS

Discovery of insulin twenty years ago served to replace almost all oral preparations of pancreas which were given in the attempt to affect carbohydrate metabolism. Wishful thinking or unwillingness to believe the overwhelming mass of testimony is still responsible for a small use of pancreatic substance orally. It is well known that occasionally oral administration of large doses of insulin will cause absorption of enough of the hormone to exert measurable effects on diabetes mellitus. The results are too erratic to be clinically important, hence the dictum that it is still impossible to use insulin other than by injection.

The improvements which have been accomplished in insulin preparations have included many steps which are of interest technically, but for the clinician the more important ones are the development of a crystalline pure substance, now known as zinc insulin crystals, and the combination of this purified insulin with such proteins as protamine in order to delay the absorption of the hormone and produce a more uniform as well as prolonged activity after injection. The differences between the standard insulin of earlier years and the zinc insulin crystals of the past three years are so slight in clinical practice that complete substitution of one for the other is possible, excepting in cases with allergic responses. There have been some diabetics who had urticaria, or occasionally other allergic sensitivity, after use of conventional preparations of insulin. The introduction of the crystalline material has been a boon for these

patients, but it offers no real benefit for the average diabetic. Due to the expiration of the patent control on the original insulin, and the continuation of such control under new patents for zinc insulin crystals, it is probable that the newer type will be used within the next few years to the exclusion of the older type. It is to be hoped that there will be no introduction into the commerce of the United States of poorly standardized or uncontrolled supplies of the older type of insulin.

The great bulk of insulin now used is the slow-acting protamine zinc insulin. The special characteristics of slow onset of action, sustained effect for about 4 days after an injection and instability requiring refrigeration and expiration dating of the packages are minor details which patient and doctor must remember. So far the precision of preparation of protamine zinc insulin and its clinical reliability have depended on the manufacture by a very few firms, which have collaborated closely with the Connaught Laboratory in Toronto, from which insulin developments have been controlled. With the introduction of zinc insulin crystals and protamine zinc insulin into the U. S. Pharmacopoeia, the control will become a responsibility subject to the offices of the U. S. Food and Drug Administration. This transition from discovery, through clinical trials, to commercial production and legal standardization has occupied just two decades. It is significant that at the same time there are in process further efforts to produce still better types of slowly acting insulin. These are not yet ready for general use.

ADRENAL MEDULLA

The introduction of the purified solution of epinephrine, the hormone of adrenal medulla, was followed by disappointment so far as replacement therapy for hormone deficiency is concerned. Until a syndrome of hypofunction of the adrenal medulla is demonstrated, we shall continue to use the hormone for its pharamcodynamic action as a sympathicomimetic drug. For this purpose it has numerous rivals due to the inventive genius of the organic chemists, especially since the re-discovery of the orally active plant alkaloid, ephedrine, which is in many ways an acceptable substitute for epinephrine. In recent years there has been a series of efforts to make the adrenal hormone have a slow and sustained action, parallel to that of protamine zinc insulin. This is being tried by combination of epinephrine with gelatine, or by solution of the epinephrine in oils from which absorption is slower than when the hydrochloride of the hormone is given in aqueous solution. This serves to introduce the entire question of the advisability of repeated injections of oils intramuscularly. The method has become common practice without rigorous study of the consequences. There are many persons who retain such oils at the site of injections for very long periods. A few people are also allergically sensitive to such oily material. The magnitude of this problem is greater in connection with the use of preparations of gonadal hormones.

ADRENAL CORTEX

Although the vital importance of adrenal cortex integrity has been recognized for a century, the preparation of life-maintaining extracts for use in Addison's disease dates back little more than a decade. In the interval since that was accomplished there has been much debate as to the type of extract which is most useful. The differences of opinion depend in large part on the fact that adrenal cortical function cannot yet be expressed in terms of one physiological process. Functions include control of the body's ability to retain sodium, to maintain life, to resist a variety of chemical and physical disturbances, and to transform protein to carbohydrate and store it as glycogen. It is still undecided whether these varied functions depend on one or more

hormone molecules, and whether or not they may all be dependent upon a single fundamental physiological activity. The isolation and identification of the pure hormone molecules involved in these processes has seemed to be a matter of a few weeks' further work—for the past year or two. There can be no doubt that the substances are of the group known as steroids, but the problem has been technically elusive and complicated by the large number of possible compounds, the lability of the active hormone and the lack of an easy and quick test for identifying the hormone.

There have been on the market in the United States at least three aqueous extracts of the adrenal cortex which will provide all the activities listed above. Whereas it would seem an easy matter to use a single process of bioassay for these competing solutions, there has been vigorous disagreement among the more experienced investigators as to the choice of a single method by which such extracts should be compared. No existing agency has been willing to appropriate enough funds to make possible the testing by unprejudiced laboratories of these rival claims, and thereby to establish the relative merits of the commercial extracts. Such a procedure would have the advantage of providing clinicians with a yardstick by which to judge the value of the materials which they must use for effective treatment of severe Addison's disease. It would do far more by establishing a definite standard for the estimation of potency of any compounds isolated from the adrenal glands and thought to be hormones and for any synthetic products thought to be identical with glandular secretions, or useful as substitutes for the natural hormone. To date such activity has been everybody's business, hence no one has been willing to undertake it. The attempts of the author to enlist the aid of non-commercial sources of support for such an undertaking have been of no avail. Yet this matter of achieving a reconciliation among investigators with regard to problems having real import for research, clinical medicine, and for commercial producers is of increasing importance in almost every phase of endocrine progress.

Until further progress can be accomplished, the clinician must rely on the commercial producers to supply dependable substances. Fortunately the differences between the extracts now on the market are not great. These materials may be injected intravenously, in cases of critical severity, using doses of 5 to 10 cc., repeated as required at intervals of a few hours. Somewhat slower and more prolonged effects may be secured by the intramuscular or subcutaneous use of 1 to 5 cc. doses, with the intervals gradually increased from 1 to 2 hours up to 1 or 2 daily doses. Since the hormones are rapidly absorbed and act for a short period, it is usually better to employ divided doses 2 or more times daily than to use a single large daily injection.

Much enthusiasm has been expressed for the synthetic steroid, desoxycorticosterone, which may be injected in oil as the acetate, or be implanted in pellets for slow absorption over a period of a few months. This substance probably occurs as a small fraction of the steroid content of the adrenals, but it is now decisively recognized that this is not the hormone of the adrenal cortex. Although the cautious use of desoxycorticosterone will substitute for the adrenals as far as life maintenance and sodium retention are concerned, the resistance to a variety of types of damage is not improved, and the ability of the body to transform protein to carbohydrate is not enhanced by the synthetic compound. Furthermore it is highly important to avoid giving an excessive amount of the desoxycorticosterone, and essential that the sodium intake be cautiously restricted under such therapy, lest there be excessive sodium retention, edema, myocardial inadequacy, hypertension and even death. This substitute for the adrenal cortical hormone extract is still safe only in the hands of trained clinicians. Its great advantage is lower cost as compared with the aqueous extracts available at present.

Another type of adrenal therapy is the use of aqueous extracts which have been converted into any one of several forms for oral administration. There is much skepticism about the oral effectiveness of cortical extracts, but their usefulness can be demonstrated in either adrenalectomized animals or patients with Addison's disease. In the latter the oral materials should not be employed during a crisis, or in any situation where nausea makes absorption undependable. Exact statements about the potency of the oral materials are more difficult to make than for the injectable extracts, for the same reasons as specified before, to which is added the uncertainty of absorption at times. The clinician who desires to use oral cortical therapy must concentrate his attention on one product, and by cautious use of this in a given patient determine the minimal effective dose.

OVARIES

For most of a century there was an attempt to use preparations of animal ovaries for substitution therapy, and this led to employment of fractions such as those from the corpus luteum, or the interstitial tissue, as well as whole ovaries, or defatted glands. The uncertain success of these methods was followed by a real therapeutic triumph when estrogenic hormone substances became available in the clinic in standardized dosage fifteen years ago. The great superiority of the newer types of ovarian materials should have eliminated the older preparations entirely by now, but there are many physicians who still cling to the ancient types and continue their demand for these pharmaceuticals, which the manufacturers would often like to discontinue. It is to be hoped that understanding of the estrogenic substances, all standardized in biological units, or in some cases in milligrams of pure hormone, will suffice at a very early date to reduce the use of the inaccurate doses of ovarian substances to the vanishing point.

Included in useful and dependable types of estrogens are the three fundamental natural estrogens, estradiol, estrone and estriol. The latter two are in large measure degradation products of the former, but all three permit the same type of substitution therapy. Estradiol is most potent per milligram, and also most rapidly inactivated in the body. All of these materials are active when injected and also when taken orally, but inactivation after oral administration is especially rapid for estradiol, least so for estriol. It is possible to use these materials orally, but the relative costs in recent years have operated against oral administration of any except estrone. Curiously, the manufacturing firms which prepare pure estrone have refused to furnish this hormone in convenient form for oral medication.

The earliest preparation of a standardized type of estrogens was a partially purified mixture of these three compounds, together with several other substances of low estrogenic activity, which are found in such sources as amniotic fluid, urine of pregnant women, or the urine of stallions or of pregnant mares. This type of non-crystalline mixture is now officially designated, according to decision of the Council on Pharmacy and Chemistry of the American Medical Association, as Estrogenic Substance, and it is standardized by comparison with estrone. This method of bioassay is not strictly satisfactory, but it provides the best approximation for the guidance of the clinician. Such material may be given in the same way as estrone, intramuscularly, intravaginally, orally, or by percutaneous absorption from ointments or from alcoholic solutions allowed to dry on the skin. There is now available on the market a growing variety of competing products of solution of estrogenic substance, prepared in oil solutions, capsules, tablets, or ointments. With improving accuracy in the bioassays it is increasingly possible to choose between these competitors on the basis of price per

unit, always keeping in mind the same route of administration when making the comparison.

It was discovered that the absorption of estradiol could be made to go on more slowly, with prolonged effects, if the hormone was given intramuscularly in vegetable oils and if the estradiol was conjugated with either benzoic or propionic acid. These esters were absorbed and used only after hydrolysis by body enzymes, with release of the free hormone. The injection in similar fashion of estrone or of estrogenic substance in these same oils is now practiced widely. Three disadvantages of this technique need to be mentioned, to offset the benefits claimed. The absorption of the hormone is by no means a uniform daily amount and steady maintenance of a given hormone concentration in the body can be maintained more readily by frequent small doses or by oral therapy than by the intramuscular injections of large amounts of the hormone in oil depots. Furthermore the oils are often found to remain for prolonged periods at the site of injection, since there are apparently no intramuscular enzymes to digest these vegetable oils and remove them. Finally, there are a few patients who have manifestations of allergic type to the administration of the oily vehicles. To avoid these difficulties there are two other methods of administration now in process of clinical trial, implantation of solid pellets, and injection of aqueous suspensions of the hormone which is almost insoluble in water. From either of these methods the solid hormones are slowly dissolved and used. The results are not yet extensive enough to justify any final statements as to their dependability.

At this stage of development there has been introduced a new approach to the therapeutic problem of estrogenic substitution in the form of the synthetic compound diethylstilbestrol. This substance is not derived from the ovarian steroids, but has certain chemical similarity to the molecular structure of the natural hormone. The compound, often spoken of by the briefer name, stilbestrol, has two great advantages: it is relatively cheap to manufacture, and it is nearly as effective if taken orally as if injected intramuscularly in oil. Following extensive study in many quarters it has been found non-toxic save for causing nausea and emesis in a minority of women and is now available for general prescription. Although diethylstilbestrol can be standardized in biological units, this is not at all necessary and it is generally prescribed in terms of milligrams. The dosage range is from 0.1 to 5.0 mg. per day for most women in the climacteric, the chief use for the estrogenic materials. It seems probable that synthetic estrogens will supplant natural ones to a large extent, although prophesy is uncertain. Further developments in this field, which can be foreseen on the basis of current experiments, involve the preparation of still better synthetic compounds and also the evolution of much higher potency dosage forms at low cost from natural sources. The balance between biological potency, therapeutic effectiveness and safety, on the one hand, and cost of manufacture and distribution on the other, makes this a rapidly changing field.

The hormone of the corpus luteum is very similar in molecular structure to those compounds just described, but its physiological activity is radically different. Its chief function is the development of the progestational endometrium, whence the hormone has been named progesterone. This substance is now available in either the natural form, crystalline pure, or partially purified; or it may be purchased in synthetic form, pure or partially purified. There is no advantage to crystalline purity of this product and this adds greatly to the cost of already expensive methods of manufacture. Therefore the progesterone preparations are assayed biologically but the standard unit is equivalent to 1 milligram of pure progesterone. We may prescribe in terms of milligram terminology. The dosages required in clinical practice are far from being

settled matters, but few experienced men expect benefit from less than 1 mg. daily, and few have used more than 5 mg. The clinical indications are apparently to substitute for or, perhaps better, to supplement the secretion of the corpus luteum. Therefore the hormone is injected after the suspected time of ovulation to increase the probability of successful nidation of a fertilized ovum or, more commonly, during the first trimester of pregnancy to reduce the chance of repeated abortions.

Progesterone is of little or no use when given orally, but recent trials of a closely related synthetic compound, pregneninolone, given orally, are of some promise. The dosage must be larger, and the certainty of clinical results is still subject to some question. It is to be hoped that further chemical study will provide compounds that can be made at low cost and yet give progestational responses when orally administered. At present, progesterone must be administered in oil intramuscularly and at short intervals such as 1 or 2 days, as long as required.

TESTES

The hormone of the testis is still being sought. There is presumptive evidence that testosterone is one of the hormone molecules and is derived from the interstitial tissue, but certainly testosterone therapy does not provide complete replacement for the endocrine activity of the testicles. Whether this means that there are two or more testicular hormones, or if merely another type of molecule remains to be found, is still comjectural. Testosterone is used as the propionate, administered in oil solution for sustained effect. Doses of less than 5 mg. per day seem of little value. Sometimes 25 mg. every day or two are used with benefit in relief of climacteric symptoms in men. The same dosage range applies to substitution in the case of eunuchs who desire to develop secondary sex characters, physical or psychic. The synthetic production of testosterone has been followed by production of a methyl ester which is much more active when administered orally. It is used in 10 mg. doses, the daily total frequently running as high as 30 mg. Results are similar clinically, although the ultimate chemical destruction of the methyl testosterone in the body is not identical with that of the simple testosterone. These materials may be used by inunction, although the advantage of this route of administration is debatable.

ANTERIOR PITUITARY

Growth promoting extracts. Stimulation of somatic growth by pituitary material depends upon the use of relatively crude extracts, which presumably represent the activity of the eosinophilic cells of the gland. These are available from several manufacturing firms, but there is no agreement on the method of bioassay to be relied upon in standardizing the extracts. Since there is also no reference laboratory to check up on these substances, no decisive statement can be made about the relative potency of any one of these preparations. At present the clinician must depend upon the word of a manufacturer whom he believes reliable. It seems obvious that there should be some impartial testing laboratory set up which would decide such matters, even in the rather early days of clinical trials. The lack of pure growth-promoting hormone materials has been allowed to hamper a working agreement among investigators on this matter of a standard test for growth promoting capacity.

Gonadotropic extracts. The most intense interest in the anterior pituitary in the past two decades has concerned the ability of the gland to stimulate the development and activity of the ovaries and testes. There is still a debate as to whether there is more than one such pituitary hormone and there are currently a number of efforts to

prepare such hormones in greater degrees of purity. These solutions always contain protein, and probably the hormone is either a protein or intimately associated with a protein molecule. The purification is, therefore, a technically difficult problem. Most authors are assuming that there is one hormone which stimulates the development and activity of the graafian follicle, and a second hormone which has to do with ovulation and the transformation of the follicle into a functional corpus luteum. With so many unsolved questions it is small wonder that no agreement has been attained on simple methods of bioassay for gonadotropic hormones. There are only a few commercial gonadotropic pituitary extracts available. These are standardized in units described by the authors whose methods a given manufacturer uses and, in general, the units of no two manufacturers are comparable. A clinician must therefore learn to use any one preparation by experience, guided by suggestions from the manufacturer.

Gonadotropic activity may be secured from the blood serum of the pregnant mare. This source provides a single substance, which has much if not all of the activity found in the anterior pituitary The gonadotropic hormone of pregnant mare's serum is not identical with the anterior lobe product, but is apparently a very useful, and at present economically competitive substitute for the gland extract. It is being produced by a few firms and fortunately there has been agreement on a method of bioassay. This material is now available, assayed in international units and compared with permanent standards set up by a special committee of the League of Nations. This substance, called equine gonadotropin, is administered hypodermically in aqueous solutions in doses of 50 to 200 units every day or two. In the case of the female, the author feels it is important that such therapy should be carried out in cycles, either interrupted after some two weeks, or at least reduced in dose, to induce cyclic function in the ovaries.

The early discovery that there is gonadotropic activity in the urine of the pregnant woman led to commercial production of what is now termed chorionic gonadotropin. This substance is entirely different from either of the other two types of gonadotropic material already mentioned. It stimulates rodent ovaries, but inhibits follicular function in primates, including the human female. The chorionic substance stimulates interstitial cell function in the testicles, and may be very useful for inducing descent of some cryptorchid testes. This is probably its chief indication in human therapy. The League of Nations Committee has also provided an international unit for this substance. The doses in general use for treatment of cryptorchidism are 100 to 500 units per day.

Posterior pituitary. The preparations of posterior pituitary are standardized by the methods prescribed in the U. S. Pharmacopoeia. This procedure applies to a liquid which is probably a mixture of an oxytocic and a pressor substance. These two substances are provided imperfectly separated, by one manufacturer only. There is infrequent use for an oxytocic which has little pressor action, and still less frequent use for a pressor fraction which lacks oxytocic activity. The chief uses of these aqueous extracts are pharmacodynamic rather than for endocrine substitution. The doses are in terms of 10 to 20 of the U.S.P. units.

For endocrine substitution posterior pituitary is useful only in cases of diabetes insipidus. Its quick and temporary action makes necessary the giving of small doses several times daily if it is injected hypodermically. Two ways have been found to avoid this inconvenience. The first is the intranasal application of a crude powder of the dried posterior gland. This can be done easily and safely, far more economically than with the sterilized aqueous extracts. The powder is a standard U.S. Pharmaco-

poeia preparation. Second, there have been recent attempts to use posterior lobe material in oil, combined with tannic acid to retard its absorption, comparable to the use of protamine with insulin. Further experience with these modifications is awaited with interest.

CONCLUSION

Clinicians are justified in asking that improved methods be set up to assure uniform potency as well as stability and dependability as to type of action of endocrine materials. The U.S. Pharmacopoeia is a legally constituted authority to publish accepted standards, but hormones will not be found in the Pharmacopoeia until difficulties in the matter of standardization have been overcome. There is no other agency which has assumed such authority. The Council on Pharmacy and Chemistry of the American Medical Association could not add this function without authority to expand its operations and also a distinctly increased budget. The Drug Division of the U. S. Food and Drug Administration serves to enforce standards which have been set up by recognized authorities. The obvious group in the United States to assume the task of bringing order out of the present confusion in the field of endocrine preparations is the Association for the Study of Internal Secretions. This group of the most interested men,—investigators, clinicians, and commercial producers—should take steps to secure the requisite financial support for a stable testing laboratory which can carry on the study of bioassay methods and recommend the standards for use. Such a project in the public interest would awaken widespread interest and confer distinct practical advantages.

PSEUDO-HYPOPARATHYROIDISM—AN EXAMPLE OF 'SEABRIGHT-BANTAM SYNDROME'

REPORT OF THREE CASES[1,2,3]

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THEORETICALLY one should obtain essentially the same clinical picture from failure of an end-organ to respond to a hormone as from a decreased production or absence of said hormone. It is the purpose of this paper to report three cases with the clinical picture of hypoparathyroidism in each of which there is evidence that the cause of the disturbance is the failure of the organism to respond to the hormone.

In our clinic at the Massachusetts General Hospital we have for a number of years used the expression, 'Seabright-Bantam Syndrome,' as a heading under which to classify those conditions in which we thought the primary disturbance was a failure of response to some hormone by the end-organ. This expression derives its origin from the fact that the male Seabright bantam has female feathering (fig. 1); the cause of this abnormality is not that the testis of the male bird produces female hormone but that the feathers of the male bird respond in an abnormal way to the normal male hormone (1, 2). Another probable example of failure of an end-organ to respond normally is met with in patients with low basal metabolic rates without other evidence of hypothyroidism. It usually requires more thyroid hormone to elevate the metabolism in these patients to normal than is required in true hypothyroidism; the inference is that the low metabolism in the first place is due to a failure of the body to react in a normal way to the thyroid hormone. Still another example is the absence of beard in the American Indian.

The diagnosis of pseudo-hypoparathyroidism in our first patient was arrived at by a lucky accident. The patient whose history is outlined as *case 1* had been treated at several Boston hopsitals for a number of years for 'idiopathic epilepsy.' She was referred to the Massachusetts General Hospital by Dr. Sarah Youngman who was impressed with the x-ray appearance of the skull. Because of the marked density of the skull the question of idiopathic hypoparathyroidism was raised. It was then found that she had a positive Chvostek sign and that her serum-calcium level was depressed and her serum-inorganic-phosphorus level elevated. The diagnosis of hy-

[1] The expense of these studies was partly defrayed by the Proctor Fund.

[2] The authors wish to thank the Winthrop Chemical Company for the generous supply of dihydrotachysterol (Hytakerol). Dr. E. J. Foley has just clarified for the authors what appeared to be a change in the strength of the marketed product. Until recently the label on the bottles indicated a strength of 5 mg. of dihydrotachysterol per cc.; more recently the label gives as the strength, 1.25 mg. per cc. The cause for the change is not that the preparation has been changed in any way, but that later studies have shown that the true dihydrotachysterol content was less than had been supposed. The discrepancy was due to an admixture of what is believed to be inert sterols. In this paper the doses refer to the older terminology.

[3] The authors also wish to thank the Eli Lilly Company for the generous supply of parathyroid extract.

Fig. 1. NORMAL SEABRIGHT BANTAM COCK. Note that tail-feathers project upwards rather than curving backwards as is usual with male chickens. (From T. H. Morgan.)

poparathyroidism seemed assured. Since the presenting symptom was epilepsy, it seemed of interest to make some careful observations on the effect of raising the blood-calcium level on that condition. It was decided to make electroencephalographic tracings before and after therapy. Furthermore, it was decided to raise the serum

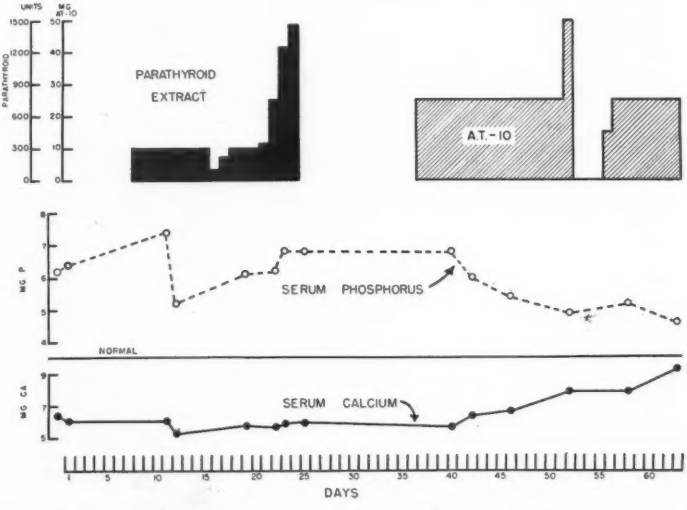

Fig. 2. DATA ON *case* 1. Note that parathyroid extract was without effect on the abnormal serum-calcium and phosphorus levels, and that A.T. 10 in large doses did affect these levels.

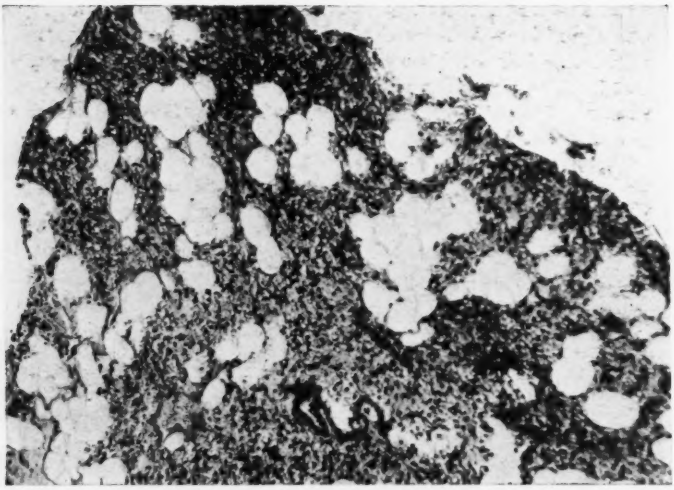

Fig. 3. BIOPSY OF PARATHYROID GLAND ON *case 1*. Note normal amount of fat tissue which is strong evidence against hyperplasia.

Fig. 4. A, *case 1*; B, *case 2*.

Fig. 5. *A*, *case 1*. *B*, *case 2*. Note roundness of faces.

calcium by the most physiological means available, namely by the administration of parathyroid extract. After the control observations had been made parathyroid extract was administered in large amounts. There was essentially no response to this medication in spite of the fact that larger and larger amounts were given (fig. 2). It was definitely established that the patient had never received parathyroid extract in the past. Had she, her resistance to it might have been accounted for. The hypothesis was arrived at, therefore, that the cause of the symptomatology was not lack of parathyroid hormone but a failure of the organism to respond to it. To help substantiate this hypothesis the patient kindly consented to a parathyroid exploration. This was carried out by Dr. Oliver Cope who had no difficulty in finding a parathyroid gland.

Fig. 6. HANDS ON *case 1*. Note shortness of fingers and fact that index fingers are longer than middle fingers.

A biopsy showed normal parathyroid tissue (fig. 3). The patient's serum-calcium level did respond to dihydrotachysterol (A.T. 10) therapy although even to this drug there seemed to be more resistance than is usual (fig. 2). With the elevation of the serum-calcium level the epileptic seizures almost disappeared.

This first patient had some rather unusual physical features. Thus, she was short and rather thick-set (fig. 4A) and had a very round face (fig. 5A); furthermore, her

Fig. 7. *Case* 3.

index fingers were longer than her middle fingers (fig. 6). The authors then remembered another patient in their group of cases with idiopathic hypoparathyroidism who had some of these same unusual physical features (fig. 4B, 5B). She was accordingly sent for and was also found to be resistant to parathyroid extract. The data on which this statement is based will be discussed in the next paragraph. For the details of the case history of this second patient the reader is referred to the case reports. It should be noted that this patient was also somewhat more resistant to dihydrotachysterol than is usual with patients with hypoparathyroidism.

The first, the most dramatic, and probably the most fundamental metabolic change following the administration of parathyroid hormone is the increase of phosphate excretion in the urine (3). Ellsworth and Howard (4) administered parathyroid extract intravenously to normal individuals and to patients with hypoparathyroidism and observed the phosphorus excretion in the urine hourly for the 3 hours before the medication and for 3 to 5 hours after medication. It was accordingly decided to carry out this exact procedure on *cases 1* and *2* to determine whether the

TABLE 1. RESPONSE TO PARATHYROID EXTRACT

Time of Day	Cases of Ellsworth and Howard: Normal Subjects						Cases of Ellsworth and Howard: Hypoparathyroid	Cases of Authors: Idiopathic hypoparathyroidism	Cases of Authors: Pseudohypoparathyroidism	
	Henry C.	Robert C.	James K.	Sam W.	Thomas S.	F.C.	Helen C.	P.R.	C.M.[1] Case 2	I.S. Case 1
	Urine Phosphorus, mg. per hour									
7–8	R16.8	5.4	12.4	18.9	20.0	29.2	0.2	11.2	9.3	1.53
8–9	9.1	7.5	11.8	24.4	23.4	32.6	0.2	20.9	12.2	4.14
9–10	12.5	12.0	8.8	28.0	21.0	43.0	0.2	15.7	8.9	4.01
	Parathyroid extract 200 U intravenously at 10 A.M.[2,3]									
10–11	42.2	56.0	48.2	42.2	47.0	113.0	11.5	313.0	14.7	9.74
11–12	51.5	69.0	42.8	61.0	lost	118.0	61.2	119.6	7.9	9.03
12–1	41.0	73.5	82.8	51.0	59.0	99.0	61.9	22.7	16.3	4.11
	Plasma Phosphorous, mg. per 100 cc.							*Serum Phosphorus mg./100 cc.*		
9 A.M.	5.3				4.8		5.8			
10:00		4.0	3.8	4.0		4.6		7.8	4.2	3.9
	Parathyroid extract, 200 U intravenously at 10 A.M.									
11:00	4.2	4.0	3.3				5.3			
12:00	4.9	4.0	3.2				5.0			
1:00		4.0	3.9	3.7	4.4	4.0	4.9	6.7	4.6	3.7
2:00	5.0									
Following A.M.	4.0	3.7	3.1	3.8	4.3	2.7	4.7		4.7	
	Phosphorus Clearance, cc. Plasma per hour							*cc. serum per hr.*		
7–8	317	135	326	470	417	635	<3.5	142	221	39
8–9	172	188	310	610	488	710	<3.5	268	271	106
9–10	236	300	230	700	431	930	<3.5	201	138	102
	Parathyroid extract 200 U intravenously at 10 A.M.[2,3]									
10–11	808	1400	1330	1055	980	2500	208	1527	334	256
11–12	1030	1725	1320	1580	670	2800	1220	1684	271	244
12–1	837	1825	2315	1360	670	2500	1250	339	193	111

[1] *Patient* C. M. received, in addition to 200 U intravenously on day of test, 500 U intramuscularly after completion of the test, and 500 U intramuscularly on the succeeding 2 days. Before starting the test serum calcium was 10.2 mg. per 100 cc. and serum phosphorus 4.2 mg. per 100 cc. Three days later, after having received 1700 U of parathyroid extract, serum calcium was 10.8 mg. per 100 cc. and serum phosphorus 4.0 mg. per 100 cc.

[2] 200 U of parathyroid extract equals 40 U of 'parathormone' used by Ellsworth and Howard.

[3] *Patient 1* received parathyroid extract at 10:30 A.M.

usual response of a phosphate diuresis to the administration of parathyroid extract was absent. A patient (P.R., M.G.H.; 4636) with the usual type of idiopathic hypoparathyroidism (5) was used as a control. In table 1 it will be seen that *cases 1* and *2* showed no phosphate diuresis or increase in phosphate clearance following the parathyroid hormone administration whereas the control care did.

The third case with pseudo-hypoparathyroidism, a boy of 3¼ years was a pa-

tient on the Children's Ward. The authors are very much indebted to Dr. Harold L. Higgins for permission to study this patient. Like *case* 1, he had had an epileptic seizure; the presenting symptom, however, was 'croup,' which turned out to be laryngeal stridor, and the diagnosis of hypoparathyroidism was made by Dr. Elliot Newman. This patient also had a positive Chvostek sign and hypocalcemia and hyperphosphatemia. His round face and stocky build (fig. 7) were so reminiscent of *cases* 1 and 2 that it seemed worthwhile to see whether he would respond to parathyroid extract. As shown in fig. 8 he did not. He too was quite resistant to A.T. 10 therapy.

DISCUSSION

It is of interest to speculate on the cause for the resistance shown by these patients to the action of parathyroid hormone. One first considers the possibility of there being in the blood antibodies ('antihormones') to the parathyroid hormone. Against this supposition is the fact that the parathyroid tissue in *case* 1 was normal rather than hyperplastic. Severinghaus and Thompson (6), by injecting pituitary

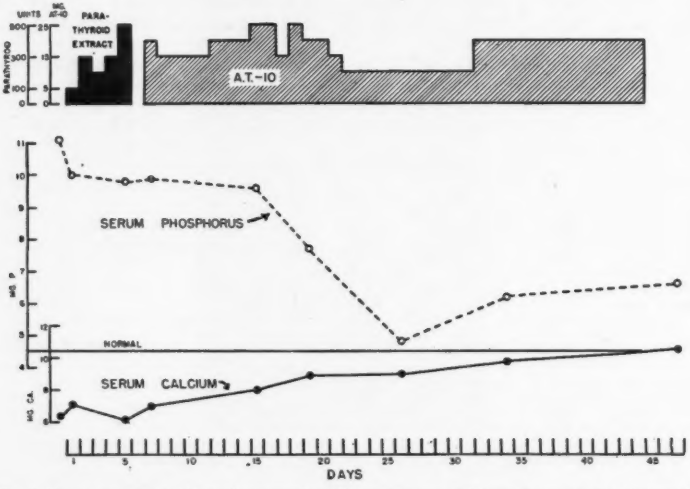

Fig. 8. DATA ON *case* 3. Note that parathyroid extract was without effect on the abnormal serum-calcium and phosphorus levels, and that A.T. 10 in large doses did affect these levels.

extracts into dogs, produced a state in these dogs wherein their own pituitary hormones were inactivated by antihormones; the ensuing clinical picture was that of hypopituitarism, and the pituitary glands themselves showed changes that were interpreted as being first a hyperactivity which was followed by an exhaustion atrophy. A second possibility is that there is a deficiency in or interference with some hypothetical substance with which the parathyroid hormone reacts. There are probably further possibilities. In any case it is little surprising that the biopsied gland in *case* 1 was not hyperplastic. Thus, one of the authors (F.A.) has always believed that the stimulus to parathyroid hormone production and hence to hyperplasia is a serum-calcium-level-below-normal. The evidence in these three cases is somewhat against this point of view. The authors are planning more experiments which, it is hoped, will throw light on the exact nature of the disturbance.

The cause for the peculiar physiognomy in these patients remains entirely obscure; it may be pure coincidence but the authors feel that it is not.

It is of interest that all three patients were somewhat resistant to A.T. 10. In a previous publication from this clinic (7) evidence was presented to suggest that A.T. 10 like vitamin D has two actions: *a*), to increase calcium absorption from the gastro-intestinal tract and *b*), to increase phosphate excretion in the urine. The second of these two actions is similar to that of the parathyroid hormone. It may be that the diminished response to A.T. 10 in this syndrome is due to an absence of this second action. The authors plan to carry out some metabolic studies on *case* 1 to study this question. The resistance to parathyroid extract and to A.T. 10 in the syndrome under discussion is quite reminiscent of the resistance to parathyroid extract and to irradiated ergosterol obtained in dogs by Taylor, Wells and Sykes (8) by prolonged parathyroid extract administration. These authors showed, furthermore, that this resistance could not be actively transmitted to normal dogs.

SUMMARY AND CONCLUSIONS

1. Three cases have been presented in which the clinical findings were those of idiopathic hypoparathyroidism but in which evidence was obtained to suggest strongly that the disturbance was not a lack of the hormone but a resistance to it.

2. The evidence that the patients were resistant to parathyroid hormone was the following: *a*). The abnormal serum-calcium and phosphorus values failed to deviate toward normal with the administration of parathyroid extract in large amounts in all three patients. *b*). *Cases* 1 and 2 failed to show a phosphate diuresis following intravenous administration of parathyroid extract. *c*). *Case* 1 was found to have normal parathyroid tissue on surgical exploration.

3. All three patients had a peculiar physiognomy characterized by a round face and a rather thick-set figure. The index fingers of *case* 1 were longer than the middle fingers.

4. None of the three patients had previously received parathyroid extract.

CASE REPORTS

Case 1, *I.S., M.G.H.* 239965, a 28-year-old female of Russian-Jewish extraction, entered the Massachusetts General Hospital on March 16, 1940 having been referred by Dr. Sarah Youngman of the Boston City Hospital. Since the age of 12 years she had been having 10 to 12 epileptic seizures daily. These had been both grand mal and petit mal in variety; there was a marked hysterical element as well. She had been followed in several other Boston clinics during this period. There had never been any carpopedal spasm. It was definitely established that she had never been treated with parathyroid extract.

The father had died 11 years previously of a shock. One sister and this sister's daughter had had epileptic seizures. The serum calcium and phosphorus levels of the sister were found to be normal. The mother and four other siblings were living and well.

The patient had had diphtheria at 4. She did not recall any other previous illnesses. The menarche had occurred at 12; the menses had been regular until 20. During the 8 years prior to admission catamenia had been very irregular occurring every 8 to 10 weeks.

The physical examination showed a short, fat, round-faced female (fig. 4A, 5A) with stubby hands and index fingers longer than the middle fingers (fig. 6). The anthropometric measurements were as follows: standing height 137.2 cm.; sitting height 76.6. cm.; span 130.7 cm. The eyes showed a high degree of astigmatism; there were no cataracts. The teeth were short and irregularly and widely spaced. The Chvostek and Trousseau signs were strongly positive. On deep inspiration the tip of the spleen was palpable. The blood pressure was 115 mm. Hg., systolic and 80, diastolic. Mentally she was extremely retarded, her mental age being placed at approximately 7 years (Bellevue scale).

Routine urine and blood studies were normal. The serum non-protein nitrogen was 14 mg. per 100 cc.; serum protein 7.2 gm. per 100 cc.; serum CO_2 combining power 25.7 milliequivalents per liter; serum cholesterol 347.5 mg. per 100 cc. An insulin tolerance test was normal. The basal metabolic rate varied from −5 to −14 per cent on three determinations. The serum calcium was 6.4 mg. per 100 cc.; serum phosphorus, 6.2 mg. per 100 cc. and the serum phosphatase 6.2 Bodansky units. In 1934 at another hospital these values had been found to be 7.8 mg. per 100 cc. for calcium and 4.0 mg. per 100 cc. for phosphorus. The hypocalcemia had not been pursued further at that time. A qualitative test for urinary calcium (Sulkowitch test) showed none present. Roentgenograms of the skull revealed marked thickening of the inner table in the frontal and parietal regions, and extensive calcification of the falx cerebrum. The bones of the hands and feet, especially the metacarpal and metatarsal bones, were extremely short and stubby. The skeleton as a whole was abnormally dense. Numerous areas of soft tissue calcification in the extremities were visible. Several of the molar teeth were unusually small with rudimentary roots. There was complete absence of several upper and lower molar teeth. The lamina dura was normal. Electroencephalograms showed abnormal waves which Dr. Robert Schwab felt were consistent with a diagnosis of epilepsy. Another encephalogram, after the serum calcium and phosphorus had become normal, showed no significant changes from the initial records.

The patient was first treated with parathyroid extract (fig. 2). She received 74 cc. (7400 U) over a period of 12 days without any definite effect upon the serum phosphorus or calcium levels. Twelve days later dihydrotachysterol (A.T. 10), 25 mg. daily, was started. On this regime her serum phosphorus gradually fell, the serum calcium rose, and calcium appeared in the urine. Coincident with these chemical changes she improved clinically. The number and severity of epileptic attacks diminished markedly. The positive Chvostek and Trousseau signs disappeared. In the ensuing months catamenia became regular.

The patient was discharged from the hospital and maintained on 5 mg. of A.T.103 times weekly. In October, 1940, she was readmitted for parathyroid exploration by Dr. Oliver Cope. At operation the right upper parathyroid gland was identified and biopsied; histological examination revealed normal parathyroid tissue (fig. 3).

The patient has been followed in the Out-Patient-Department. On 5 mg. of A.T. 10 three times weekly her serum phsophorus and calcium have been maintained at relatively normal levels; and although she still has epileptic seizures, they are less severe and much less frequent.

Case 2, C.M., *M.G.H. 149639*, a 12-year-old American female, entered the Massachusetts General Hospital on March 23, 1939 because of attacks of tetany. At the age of 3 she began to have attacks of carpopedal spasm. After a tonsillectomy at 4 she had no more pedal spasm until just before her admission to the Massachusetts General Hospital. Attacks of carpal spasm, however, had continued to recur 2 to 3 times yearly. There was no history of convulsions. Treatment had consisted of oral and parenteral administration of various calcium salts. In January, 1939, the patient had an attack of both carpal and pedal spasm and she was referred to the hospital by Dr. John Boardman.

The past history revealed that the patient's birth and infancy were normal. At the age of 4 she had whooping cough. The parents stated that she had always been somewhat large and overweight for her age, and they felt that she was somewhat backward mentally.

The family was American. The parents were healthy. Two siblings, aged 15 and 13 years, were normal. No familial diseases were known.

Physical examination revealed a moderately obese, round-faced young female (fig. 4B, 5B). There were no cataracts. The teeth showed blunted root ends, retarded eruption and a hypoplasia of the enamel similar to that which has been observed in other cases of hypoparathyroidism (9). The Chvostek and Trousseau signs were positive. The thyroid gland was just palpable and firm. The blood pressure was 105 mm. Hg, systolic and 65, diastolic.

Routine blood and urine studies were normal. The Hinton test for syphilis was negative. A basal metabolic rate was −11 per cent. The serum non-protein nitrogen was 14 mg. per 100 cc.; serum protein 7.2 gm. per 100 cc.; serum calcium 8.9 mg. per 100 cc.; serum phos-

phorus 6.3 mg. per 100 cc.; serum phosphatase 7.1 Bodansky U. No calcium was present in the urine by qualitative test. Roentgenograms of the skull and pelvis showed increased density. The teeth showed marked shortness of all premolars with tapering but blunted root ends. Many of the crowns showed a fuzzy irregularity. Several teeth were unerupted. The lamina dura about the teeth was normal. The epiphyses of the right wrist and elbow were normal for the patient's age.

The patient was started on dihydrotachysterol, 5 mg. daily and discharged from the hospital. However, after one month on this regime she still had signs of tetany, and there was no calcium in her urine by rough test. By increasing the dosage of A.T. 10 to 10 to 15 mg. daily during the next 2 months calcium finally appeared in the urine and all signs of tetany disappeared. Subsequently it was found that 5 mg. daily were necessary as a maintenance dose. In October, 1940 her serum calcium was 10.3 mg. per 100 cc.; serum phosphorus 3.5 mg. per 100 cc. Sulkowitch test of the urine revealed a 1 + calcium.

Because of the patient's physical resemblance to the other two cases she was asked to return for an Ellsworth-Howard test on April 22, 1941. At the time of this test she had taken no A.T. 10 for 3 weeks. The results (table 1) were discussed above. In addition to the 200 U of parathyroid hormone given intravenously on April 22, she was also given 500 U intramuscularly later in the day, and 500 U intramuscularly on April 23 and 24, respectively. On April 22, before receiving any parathyroid hormone the serum calcium was 10.2 mg. per 100 cc. and the serum phosphorus 4.2 mg. per 100 cc. On April 25 the serum calcium was 10.8 and the serum phosphorus 4.0 mg. per 100 cc. Hence she received 1700 U of parathyroid hormone in 3 days without any effect upon her serum-calcium or phosphorus levels.

Case 3, *F.M., M.G.H. 287678*, a male of 3 years and 3 months, first entered the Massachusetts Eye and Ear Infirmary on Feb. 1, 1941 because of 'choking spells.' His parents stated that at the age of 2 years he developed wheezing respirations, and began to have attacks of choking during which he was apparently struggling for air. At the age of 2 years and 6 months he had several convulsions. About this time his physician observed a laryngeal 'paralysis.' A tonsillectomy was performed, and he became relatively asymptomatic until the age of 3 years and 2 months when the respiratory symptoms recurred. There had been no carpopedal spasm.

The patient's birth and early infancy were normal. He had uncomplicated measles at 21 months and uncomplicated pertussis at 24 months. He had always been somewhat obese. He had never talked although he seemed normally intelligent.

The family was American. Both parents were healthy. Two siblings, aged 1 year and 5 years, were normal. There were no familial diseases.

Physical examination showed a stocky, round-faced child (fig. 7). The anthropometric measurements were as follows: standing height 36 inches; sitting height 21 inches; span 33 inches. There were no cataracts. The deciduous teeth were normal. Respirations were wheezing and there was definite laryngismus and stridor. On direct laryngoscopy the vocal chords were found to occupy an almost mid-line position on inspiration. These findings were felt to be due to an abductor paralysis. Small, hard, calcified masses were felt in the subcutaneous tissues of the trunk and extremities. The patient was seen in consultation by Dr. Elliot Newman who made a diagnosis of hypoparathyroid tetany with laryngospasm, and had him transferred to the Children's Medical Service.

Routine urine and blood studies were normal except for a moderate leucocytosis. The Hinton test was negative. The serum non-protein nitrogen was 26 mg. per 100 cc.; serum protein 6.8 gm. per 100 cc.; serum total cholesterol 204 mg. per 100 cc.; serum chlorides 99.3 m.eq. per liter; serum carbon dioxide content 25.4 m.eq. per liter; serum total base 159.5 m.eq. per liter. The serum calcium was 7.1 mg. per 100 cc.; serum phosphorus 10.0 mg. per 100 cc. and serum phosphatase 8.7 Bodansky U. Roentgenograms of the skull and long bones revealed no abnormalities. The deciduous teeth were all present. The development of the permanent buds was slightly precocious, but no changes characteristic of hypoparathyroidism were present. Areas of calcification were visible in the soft tissues of the chest, abdomen and extremities.

The patient received 1700 U of parathyroid hormone between Feb. 7 and 12, 1941, without any effect on serum calcium or phosphorus (fig. 8). Dihydrotachysterol was the started on Feb. 13, 1941. About this time he developed an upper respiratory infection and his laryngospasm became so severe that it was necessary to perform a tracheotomy on February 25. He gradually responded to A.T. 10, but it was found that rather large doses were necessary to raise his serum calcium to normal levels (fig. 8). After the serum calcium became normal, direct laryngoscopy revealed that the vocal chords were still in the position of abductor paralysis. Hence it was felt unsafe to close the tracheotomy opening, and he was discharged from the hospital on 5 mg. of A.T. 10 daily.

In December, 1941, the patient showed no signs of tetany. He was growing rapidly and beginning to talk. The serum calcium was 9.4 mg. per 100 cc. The tracheotomy tube was still in place but the vocal chords now definitely moved and it was hoped that it would be possible to remove this soon.

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THE ESTROGENS

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PERHAPS in reviewing progress in the estrogen field since the founding of the Journal, ENDOCRINOLOGY, it is appropriate to direct attention to a publication which appeared in the same year that the new Journal was launched and which led to the bioassay method upon which many of the chemical and biological advances have depended. In 1917, Stockard and Papanicolaou (1) showed that the type of cells in the vaginal smear could be correlated with the phases of the sex cycle of the guinea pig. Subsequently, Long and Evans (2) and Edgar Allen (3) reported that a similar correlation prevailed in the rat and mouse, respectively. Upon his study of the mouse, Allen (4) based his well-known method of detection and assay of estrogenic activity which has played a highly important rôle in the investigations of the estrogens.

Using this convenient method of assay, the earlier reports of the existence of uterine growth stimulants in the placenta (Fellner, 5) and the liquor folliculi (Frank, 6) were confirmed and rapidly extended, but the next fundamental contribution to the isolation of the estrogens was the discovery of the high concentration in the urine of pregnant women (Aschheim and Zondek, 7) and mares (Zondek, 8). The importance of these discoveries is obvious from the rapidity with which the isolation was accomplished.

ISOLATION OF ESTROGENS

The first pure crystalline estrogen, estrone, was obtained independently and almost simultaneously by Doisy (9) and Butenandt (10) in 1929 from the urine of pregnant women. Since the chemical nature of the new compound was not known, the purification had been guided step by step by bioassays. After isolation had been accomplished and the properties of the new compound determined, much more satisfactory methods of purification were devised.

The methods now used are based on the conversion of the conjugated water-soluble compound by hydrolysis to the ether-soluble free phenol, extraction with an immiscible solvent (*e.g.*, ethyl ether) and separation of the phenolic from the neutral and acidic fractions. The purification of estrone present in the phenolic fraction can be facilitated by the use of Girard's *T* reagent (11), trimethylacethydrazide ammonium chloride, or, carboxymethoxylamine, a ketone reagent synthesized by the method described by Borek and Clarke (12) and first used in the estrogen field by Wintersteiner (13).

The isolation of estrone was followed less than one year later by the separation in pure form of another crystalline estrogen, estriol, from the urine of pregnant women (Marrian, 14; Doisy, 15). Shortly thereafter Laqueur (16) accomplished the isolation of estrone from the urine of pregnant mares and in 1932 Girard (17) obtained three new estrogens, equilin, equilenin, and hippulin from the same source. The isolation of these and still other estrogens from biological sources is summarized in table 1.

TABLE 1. ISOLATION OF ESTROGENS

Compound and Source	Date	Investigator
Theelin or Estrone, $C_{18}H_{22}O_2$		
Urine, pregnant women	1929	Doisy, Veler and Thayer (9); Butenandt (10)
Urine, pregnant mares	1931	DeJongh, Kober and Laqueur (16)
Urine, men	1938	Dingemanse, Laqueur and Muhlbock (18)
Urine, stallion	1934	Haussler (19); Deulofeu and Ferrari (20)
Placenta, human	1938	Westerfeld, MacCorquodale, Thayer and Doisy (21)
Testes, stallion	1940	Beall (22)
Adrenal, beef	1939	Beall (23)
Palm kernels	1933	Butenandt and Jacobi (24)
Estrone sulfate		
Urine, pregnant mares	1938	Schachter and Marrian (25)
Theelol or Estriol, $C_{18}H_{24}O_3$		
Urine, pregnant women	1930	Marrian (14); Doisy, Thayer, Levin and Curtis (15)
Placenta, human	1931	Browne and Collip (26)
Pussy-willows	1933	Skarzynski (27)
Estriol glucuronide		
Urine, pregnant women	1935	Cohen and Marrian (28)
Dihydrotheelin or α-Estradiol, $C_{18}H_{24}O_2$		
Ovaries, sow	1935	MacCorquodale, Thayer and Doisy (29)
Urine, pregnant mares	1935	Wintersteiner, Schwenk and Whitman (30)
Urine, pregnant women	1939	Smith, Smith, Huffman, MacCorquodale, Thayer and Doisy (31)
Placenta, human	1940	Huffman, Thayer and Doisy (32)
Testes, stallion	1940	Beall (22)
β-Estradiol, $C_{18}H_{24}O_2$		
Urine, pregnant mares[1]	1937	Hirschmann and Wintersteiner (33)
Equilin, $C_{18}H_{20}O_2$		
Urine, pregnant mares	1932	Girard, Sandulesco, Fridenson and Rutgers (17)
Hippulin, $C_{18}H_{20}O_2$		
Urine, pregnant mares	1932	Girard, Sandulesco, Fridenson and Rutgers (17)
Equilenin, $C_{18}H_{18}O_2$		
Urine, pregnant mares	1932	Girard, Sandulesco, Fridenson, Gaudefroy and Rutgers (17)
17-Dihydroequilenin, $C_{18}H_{20}O_2$		
Urine, pregnant mares	1936	Wintersteiner, Schwenk, Hirschmann and Whitman (34)
Δ-5,7,9-Estratrienol-3-one-17, $C_{18}H_{22}O_2$		
Urine, pregnant mares	1940	Heard and Hoffman (35)

[1] Confirmed in my laboratory by E. A. Doisy, Jr., and S. A. Thayer.

Several interesting points are suggested by examination of table 1. Although in some instances explanation is not yet possible, still attention should be called to these points of uncertainty.

Estriol is the principal estrogen of human pregnancy urine but thus far it has not been obtained from mare urine. On the other hand, mare pregnancy urine contains at least seven estrogens of which five have not been found in human urine. Perhaps, these differences signify different courses of estrogen metabolism in these species.

The occurrence of the more highly unsaturated estrogens in mares' urine is also interesting from the viewpoint of their relationship to estrone. Are they to be regarded as products of metabolism or of intermediates in the synthesis of estrone? Furthermore, it is curious that they have not been found in the urine of stallions. Thus far only estrone has been found in the urine and estrone and α-estradiol in the testis of the stallion but the presence of even these estrogens leads one to wonder about their possible physiological importance in the male.

The occurrence of estrone in beef adrenals is also a mystery. Is it synthesized or merely stored in that gland? Certain evidence does seem to indicate the formation of estrogens in the adrenal.

Perhaps, it is hardly fair to pose such questions but they have been troubling the author for sometime.

Isolation of estrogens from human placenta, and other sources. The isolation of estriol from human placenta (Browne, 26) was the first instance in which a pure estrogen was obtained from sources other than urine. Subsequently, two additional estrogens, estrone (Westerfeld, MacCorquodale, Thayer and Doisy, 21), and α-estradiol (Huffman, Thayer and Doisy, 32) were isolated from the same source. All three of these estrogens have likewise been obtained from the urine of pregnant women. Although β-estradiol has been isolated from pregnant mares' urine and from the urine of rabbits (Fish and Dorfman, 36; Heard, Bauld and Hoffman, 37) following the administration of α-estradiol and estrone, thus far this compound has not been obtained from human sources.

With the exception of those from sow ovaries, the nature of the estrogens in ovarian tissue has received little attention. After an extensive investigation, in which

TABLE 2. COMPARATIVE YIELDS OF ESTROGENS

Source	Ketonic	Non-Ketonic	
	estrone	*α-estradiol*	*estriol*
Sow ovary	0.010 mg. per kilogram	0.014 mg. per kilogram	?
Human placenta	0.035 mg. per kilogram	0.038 mg. per kilogram	0.140 mg. per kilogram
Human pregnancy urine	1–3 mg. per 24-hour specimen	0–0.6 mg. per 24-hour specimen	10–30 mg. per 24-hour specimen

the liquor folliculi of about four tons of sow ovaries was extracted, a few milligrams of a crystalline compound which was identified as α-estradiol was isolated. Subsequently, Westerfeld, et al. (38) examined extracts of sow ovaries from which most of the liquor folliculi had been removed and found a ketonic estrogen. It was not isolated but evidence clearly indicated that it was estrone.

A few remarks on the quantities of estrogens in certain sources might be of interest. In their work on sow ovaries, MacCorquodale, et al. (29) obtained about 6 mg. of α-estradiol from the liquor folliculi of a ton of ovaries but found only traces of a ketonic estrogen. However, in the later work on whole ovaries from sows and also sow ovaries from which the liquor folliculi had been removed, Westerfeld (38) obtained results which indicated approximately equal quantities by weight of ketonic and non-ketonic estrogens. The accumulated evidence indicated that the ketonic estrogen was largely if not entirely estrone.

The assay of purified fractions of human placenta shows that this tissue contains somewhat larger quantities of both estrone and α-estradiol than are found in sow ovaries and in addition contains estriol (see table 2). On a weight basis the amount of estriol in placenta is double the total weight of estrone and α-estradiol.

Although α-estradiol was isolated from human pregnancy urine in 1939, very few quantitative data on its occurrence are available. Those listed in table 2 were obtained by inspection of the reports published by Smith and Smith, and Bachman (39). Though the values for the individual estrogens may show wide variations, it is evident that estriol is excreted in quantities much larger than those for estrone and estradiol.

In a previous paper (40) a method which was based on Mather's (41) partition ratios and Huffman's (42) study of carboxymethoxylamine was described for the separation of estriol, α-estradiol and estrone. Since β-estradiol has been found in the urine following the administration of estrone and α-estradiol (Stroud, 43, Fish and Dorfman,

36, Heard, Bauld and Hoffman, 37), it was necessary to ascertain where β-estradiol fitted into this scheme. It was found that this compound appears in the α-estradiol fraction (Van Bruggen, 44). Thus far, a satisfactory method for the separation of small amounts of these compounds has not been found. Of course, for larger quantities, the precipitation of the α-estradiol with digitonin (Wintersteiner, 45; Butenandt, 46) is available.

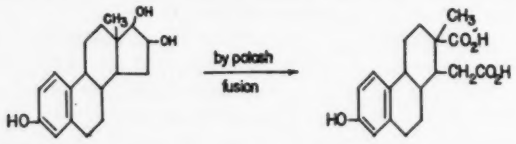
The finding of β-estradiol in the urine of animals should lead to a search of human urine for this compound.

Conjugated estrogens and hydrolysis. Hydrolysis of conjugated estrogens continues to be a vexing problem. Three methods (47) or modifications of them have been used rather extensively but it is generally admitted that destruction occurs during hydrolysis and the amount of estrogen finally obtained is less than the total estrogen originally present.

According to Beall and Marrian (48) the hydrolysis of the conjugated estrogens of mares' urine is effected by a procedure less vigorous than that required for human urine. Since estrone sulfate appears to be the conjugation product of the mare (25), and since Butenandt (49) found that this compound is easily hydrolyzed, it seems likely that estriol glucuronide is the product which causes the difficulty in the hydrolysis of human urine.

CONSTITUTION OF THE ESTROGENS

The isolation of a new compound affords the opportunity to determine its structure. The final step in the determination of constitution is synthesis and comparison of the synthetic with the natural product. In the case of the estrogens the structure indicated by degradation experiments has been confirmed by the total synthesis of equilenin by Bachmann (50) and since Marker (51) had already converted α-dihydroequilenin to α-estradiol, the structure of estrone is of course established.



Estriol

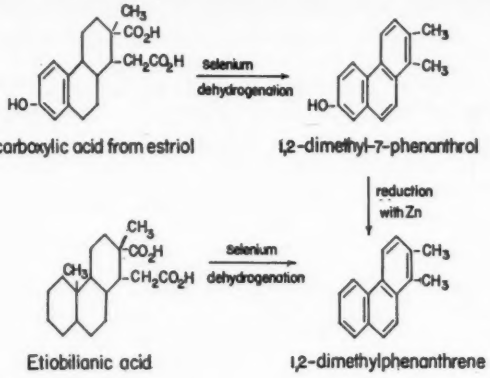
The early work of Butenandt (52) and the St. Louis group (53) established $C_{18}H_{22}O_2$ as the formula for estrone. It was shown that estrone contains phenolic and ketonic groups. Since estriol contains a phenolic group (Marrian, 54; Doisy, 55) and two additional hydroxyls, Butenandt (56) heated the compound with $KHSO_4$ and obtained estrone by the abstraction of one mole of water. This indicated identity of ring structures and since estriol was much more abundant than estrone at that time, it was used for the next work on structure. By potash fusion both Marrian (57) and MacCorquodale (58) obtained a dicarboxylic acid of eighteen carbon atoms from estriol and from their evidence concluded that the two hydroxyls of the original compounds were attached to adjacent carbon atoms contained in a five-membered ring.

X-ray and crystallographic studies by Bernal (59) indicated that the hydroxyl and carbonyl groups are at opposite ends of the molecule. The mono-molecular film data obtained by Adam and Danielli (60) were consistent with a condensed four ring system in which one of the rings is aromatic, though other possibilities were not excluded.

Butenandt (61) dehydrogenated the dicarboxylic acid previously mentioned with powdered selenium, reduced the product with zinc dust and obtained dimethylphen-

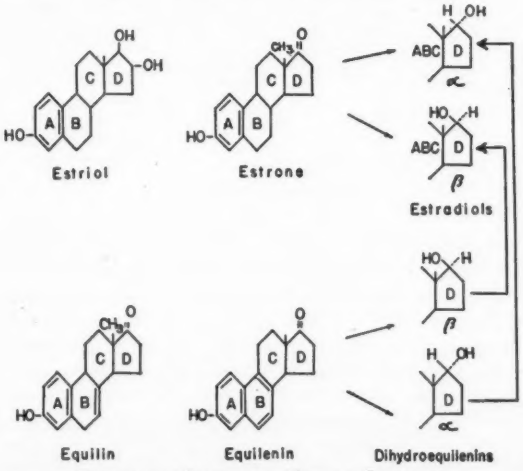
anthrene. The same compound was obtained from etiobilianic acid, thereby showing a similarity between the ring systems. Synthetic work clearly showed that the aromatic hydrocarbon which had been obtained was 1,2-dimethylphenanthrene.

On the basis of the available evidence, Butenandt suggested that estrone has a condensed ring system similar to that occurring in sterols. In his formulation, ring A



was aromatic, the phenolic hydroxyl was placed at carbon atom 3 and the other oxygen atom was attached to carbon atom 17 (see formulae below). Subsequent investigation proved this formula to be correct.

In 1934, Haworth and Sheldrick (62) synthesized 1,2-dimethyl-7-methoxyphenanthrene which upon comparison with the methyl ether of Butenandt's degradation



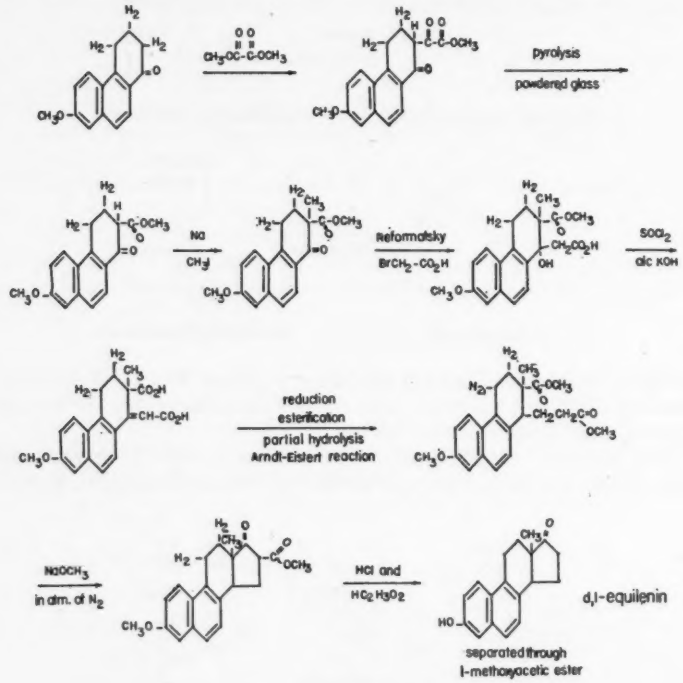
FORMULAE OF SOME OF THE NATURAL ESTROGENS

In the upper half of the diagram, the formulae differ only in the substituents of the D ring. The representation indicates that the A, B, and C rings are identical for estrone, estriol and the estradiols.

In the lower half of the diagram, the structure of the B rings differs from the B ring of estrone; furthermore, equilin and equilenin differ in the B ring. The arrows at the right hand side indicate that the α and β dihydroequilenins can be converted by reduction into the α and β estradiols, respectively. The ring structure of dihydroequilenin and equilenin is the same.

product from the dicarboxylic acid proved to be identical. This established the position of the hydroxyl group on carbon atom 3 in estrone (position 3 in estrone is identical with position 7 if estrone is named as a derivative of phenanthrene) and the aromatic nature of ring A.

In Cook's (63) laboratory the position of the hydroxyl was also established by synthesis of 7-methoxy-1,2-cyclopentenophenanthrene which proved to be identical



BACHMANN'S SYNTHESIS

with a compound prepared from methoxyestrone by reduction of the keto to a methylene group and subsequent dehydrogenation. The same product was obtained from equilenin, thereby giving assurance of the similarity of the ring structures of estrone and equilenin. Further work by Cook (64) and his associates established that the oxygen atom in estrone is at position 17 and the angle methyl group at position 13. The synthesis of equilenin by Bachmann fully confirmed the prevailing view of the structure of the estrogens.

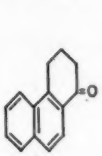
SYNTHESIS OF NATURAL ESTROGENS

Bachmann's (50) splendid synthesis of equilenin—incidentally the only total synthesis of a sex hormone—merits thorough discussion but unfortunately that is not possible in this short review. Starting with 7-methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene, which had been obtained from 1-naphthylamino-6-sulfonic acid, *d*-equilenin and its three isomers were synthesized. Each step of the synthesis as depicted above, was studied until yields of 90 per cent or more were obtained.

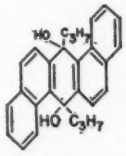
Since Marker (51) had already converted α-dihydroequilenin to α-estradiol, Bachmann's synthesis firmly established the structure of both equilenin and estrone.

SYNTHETIC ESTROGENS WITHOUT THE PERHYDROCYCLOPENTENO-PHENANTHRENE NUCLEUS

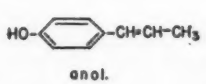
The discovery of so many natural estrogens suggested a lack of specificity and that perhaps compounds without the cyclopentenophenanthrene nucleus might possess estrogenic activity. In their early work, Dodds (65) and his associates tested phenanthrene derivatives and found that 1-keto-1,2,3,4-tetrahydrophenanthrene possessed a slight degree of activity. Derivatives of dibenzanthracene were shown to be somewhat more active (66), the most potent compound being 9,10-dihydroxy-9,10-di-n-propyl-9,10-dihydro-1,2,5,6-dibenzanthracene. Continuing their work, simple phenols containing only a single ring were studied (67). In the hydrolysis of anethole



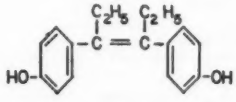
1-Keto-1,2,3,4-tetrahydrophenanthrene



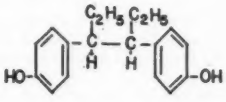
9,10-Dihydroxy-9,10-di-n-propyl-9,10-dihydro-1,2,5,6-dibenzanthracen



anol.



Diethylstilbestrol



Hexestrol

the anol which was produced occasionally showed high potency. Subsequently, this was found to be due to an impurity, probably produced by the reaction between two molecules of anol. In collaboration with Robinson (68) an extensive series of related compounds was prepared for assay and the highly potent 4,4'-dihydroxy-α,β-diethylstilbene (stilbestrol) discovered. The compound which had been present in the anol as an impurity was isolated and identified as hexestrol (69), a compound which can be obtained by reduction of stilbestrol.

DISCUSSION AND SUMMARY

Although many important problems in medical science still baffle investigators, it is encouraging to review occasionally the progress that has been made in an active field in which important results have been achieved. In each of these unsolved problems someone will take the right turn or make the unique and fundamental discovery which will lead to the correct solution.

In the estrogen field the turning point came only twenty-five years ago with the study of the sex cycles of the guinea pig, rat and mouse and then somewhat later Edgar Allen's recognition of the value of vaginal smears for assay facilitated work on the 'follicular hormone.' The chemical work was expedited by the discovery by Asch-

heim and Zondek of the high concentration of estrogens in pregnancy urine. Making use of these important contributions the isolation of pure estrogenic compounds was rapidly accomplished. Determination of the structure posed a difficult problem in synthesis which taxed the skill of organic chemists but which in a few years yielded to Bachmann's attack. In the meantime, other unrelated compounds which have high estrogenic potency were synthesized.

Thus, in the short period of the existence of this Journal, a mysterious and complex problem has been solved by the combined contributions of scientific workers.

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ESTROGENIC HORMONES IN THE GENESIS OF TUMORS AND CANCERS[1]

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THIS paper cites a few examples of tumors and cancers occurring in experimental animals after long-continued treatment with estrogenic hormones.[2]

1. Cancer of the cervix uteri in mice
2. Uterine fibromyomas in guinea pigs
3. Mammary cancer, especially in mice
4. Interstitial cell tumors of the testis in mice
5. Adenomatous tumors of the pituitary in rats and mice
6. Tumors of the suprarenal cortex in mice. (These may occur spontaneously after ovariectomy, with evidence that the tumor secretes estrogen)
7. Osteogenic transformations of bones, including tumors in mice

The following conditions, classed here as atypical growths, have been reported following excessive estrogenic treatment, or gonadotropic treatment in the case of cystic ovaries.

1. Cystic hyperplasia of the endometrium in several species
2. Metaplastic changes in parts of the male accessory organs
3. Leukemia, lymphatic; (as lethal in mice as in man)
4. Hypertrophy of the bile ducts in mice
5. Cystic ovaries in several species

Genital organs have proved most reactive (1, 2), but growths of nongenital tissues also appear, such as leukemia, osteogenic changes and tumors, and hypertrophy of the bile ducts.

Several estrogens have returned positive tests (3), including estrone, estriol, estradiol and its benzoates, propionates and other compounds, equilin and equilenin and their benzoates and diethylstilbestrol. All estrogens seem to have carcinogenic properties although their potencies vary.

Androgens in experimental animals have been factors in excessive growths or hypertrophies (4) but are not regarded highly as agents in tumorigenesis. Androsterone and testosterone, their propionates and other combinations have been tried. However,

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[1] Grateful acknowledgment is made to The Anna Fuller Fund, The International Cancer Research Foundation, and The Jane Coffin Childs Memorial Fund for Medical Research, for the support of much of the work cited from this laboratory.

[2] More thorough reviews, citing many of the details of the experimental evidence, will be found in the following: "Estrogens in carcinogenesis." W. U. GARDNER, *Arch. Path.*, 27: 138. 1939; "A survey of compounds which have been tested for carcinogenic activity." J. L. HARTWELL, *National Cancer Institute, U.S.P.H.S.* 1941, which contains a long bibliography; "The significance of hormones in the origin of cancer." L. LOEB: *J. Nat. Cancer Inst.* 1: 169. 1941; "Biology of the Laboratory Mouse." By the Staff of the Roscoe B. Jackson Memorial Laboratory, P. Blakiston's Sons, Philadelphia, 1941.

when injected simultaneously with estrogens in mammary tumor-susceptible females, androgens reduce the incidence of mammary cancer (5). Progesterone has not been proved carcinogenic as yet, nor has pregnandiol. Desoxycorticosterone has returned negative tests (6).

Gonadotropic factors induce remarkable effects, especially in the ovaries, where cystic tumors can be induced and maintained for some time by continued injections. When gonadotropic stimulation is withdrawn, ovarian conditions may return to normal. Experimentally induced imbalance in pituitary function, by parabiosis (7) or testis transplants (8), may prevent ovulation and be followed by cystic ovaries of the follicular type. Estrogen secreted by such cystic ovaries may induce hypertrophic conditions in the uterus similar to those experimentally induced by excessive estrogenic treatment (8, 9). These later experiments show that endogenous estrogens may be effective in producing atypical growths. Exploration of the possible rôle of gonadotropins in testicular tumors offers much promise (10).

1) *Cancer of the cervix uteri.* This is recognized as an extremely lethal lesion in women, and difficult to diagnose with certainty in early stages.

Cancer of the cervix of mice following chronic injection of estrogen was reported in 1936 (11, 12, 13). Since the mouse has no mucous glands in the cervix, but instead a low stratified epithelium extending throughout the cervical canal, the cancers of the cervix are squamous cell carcinomas. It usually requires at least a year's treatment with estrogen before cervical cancer appears. In mice, which are susceptible to mammary cancer, mammary cancer may develop before lesions of the cervix, and in some of the early experiments it was necessary to remove large mammary cancers so that the animals might live long enough for cervical cancers to develop (14). In extreme cases (*Stage IV* lesions) the cancer may invade the muscles of the rectum and bladder, almost fill the pelvis, and occasionally protrude from the vagina. All intermediate stages in the development of these extreme lesions have been obtained. Now that this type of cancer can be produced experimentally the earliest stages of infiltrating lesions can be studied. Frequently one finds several points of invading growth of the epithelium, indicating that in these experimental animals the lesions need not start from a single focus.

It was early noted that estrogenic treatment was followed by development of cervical cancer in inbred strains of mice that were resistant to mammary cancer. The only complicating difficulty was that mice resistant to mammary cancer had very poor tolerance of the chronic estrogenic treatment at levels effective for cervical carcinogenesis (15).

When the work was extended from pure strains to hybrids, and so planned that the hybrid would tolerate estrogenic treatment, experiments reported recently recorded an incidence of 50 per cent of cervical cancer in one group and 62 per cent in another group of such hybrids which survived estrogenic treatment for more than one year (16).

It was possible to study the relation of the 'milk factor' to cervical cancer in the hybridization experiment, and it was shown that the nursing, so important in determining the incidence of mammary cancer, has apparently no relationship to the incidence of cancer of the cervix which follows estrogenic treatment.

Two other points should be noted. Cervical cancer, unlike mammary cancer, does not appear spontaneously in mice. In some cases the cancerous tissue was proven autonomous in transplants to both males and females of the same strain (14). Continuous growth of the transplants does not require continued estrogenic treatment of the hosts, as is required for successful transplants of interstitial cell tumors of the

testis. Simultaneous injections of androgens or progesterone do not modify the incidence of cervical cancer following estrogenic treatment.

2) *Uterine fibromyomas.* Uterine fibroids in women are usually benign but may grow to large proportions and be serious as obstructions. They occur in a high incidence in negro women. They have been associated with cystic ovaries or other evidences of unbalanced or excessive ovarian secretion (17) although other factors may be involved also.

Though thousands of mice have been subjected to chronic estrogenic treatment, their uteri do not develop fibroids. Experimentally induced fibromyomas first appeared in the uteri of guinea pigs after 6 to 8 months of daily treatment with large doses of estrogen (18). Muscular as well as fibrous tissue was involved. The tumors were often multiple, appearing as button-like growths under the peritoneum. The question of their continued growth after cessation of hormone treatment should be tested further. Somewhat similar fibroid growths may appear on mesenteries and intestinal peritoneum of both male and female guinea pigs after relatively short treatment with estrogens (19). These growths are usually not malignant and they retrogress when treatment is stopped. They may be transplanted and will grow in the hosts only if the hosts are injected with estrogen.

The special susceptibility of the guinea pig to the growth of fibroids seems at present as clearly a species limitation as the high incidence in Negroes seems racial.

3) *Mammary cancer.* The best early evidence for participation of internal secretions from the ovary in mammary cancer was the demonstration that ovariectomy at early ages prevented or greatly reduced the incidence of spontaneous mammary cancer in strains of mice which normally had a high incidence (20).

The next milestone in the attack of this problem was the experimental production of mammary cancer in male mice following injections of estrogen (21). The males must be of strains whose females have a high incidence of mammary cancer. Before this time many experiments had shown that estrogens induced growth in mammary rudiments and one explanation of Lacassagne's experiments was that genetic factors for cancer, present in males as well as in females, became operative only after the mammary glands had grown in response to estrogenic stimulation.

Later, it was established that strains of mice in which the females normally had a high incidence of mammary cancer, had abnormal mammary glands even before cancer was recognizable (22). The abnormality consisted of several or many hyperplastic nodules of alveolar tissue interspersed through an otherwise normal mammary system. Continued hyperplasia in these nodules was especially noticeable when the rest of the glands began involution with the approach of old age. When a small cancerous nodule becomes palpable, its origin from one of these nodules of an atrophic gland seems assured, for all intermediate stages occur in a finely graded series. Rapid growth of that nodule into a cancer lethal in a few weeks seemed to inhibit the growth of other nodules. Removal of the rapidly growing one is usually followed shortly by the appearance of cancerous nodules in other regions of the same gland or other glands of the same animal.

Then it was shown that injections of estrogens into female mice, normally low in incidence of spontaneous mammary cancer, was followed by a marked increase in the incidence of cancer in those strains (23). This might be interpreted to indicate that the estrogenic hormone intensified or augmented the genetic factor for mammary cancer.

Various estrogens have been shown to be effective, although there is some variation in potency. Estrone (theelin) estriol (theelol) equilin, equilin benzoate, hydroxy-

estrin benzoate, ketoestrin benzoate and diethylestilbestrol, all have proven effective (3). Estrogens which occur normally in the body and those which are normally foreign are both effective.

While small doses of estrogen induced normal growth of the mammary rudiments, they were definitely below the level of stimulation effective for mammary cancer. Larger doses were required, but massive doses were shown to result in stunted or atypical growths (24). It is possible to carry this stunted growth of the glands to extremes and even prevent growth of the mammary rudiments by large doses of the same hormone which is effective in lesser amounts.

While the importance of estrogen and other endocrine factors was under investigation, the maternal or 'milk factor' in mammary carcinogenesis was demonstrated (25, 26). Briefly stated, if young from mammary cancer-susceptible mothers are foster-nursed by cancer-resistant mothers, the incidence of mammary cancer is reduced. Incredible though it may seem, a nursing period restricted to 1 or 2 days may be enough to be followed by the appearance of mammary cancer 10 or 12 months later. Conversely, young from strains resistant to mammary cancer foster-nursed by susceptible mothers have a slightly increased incidence of mammary cancer. This work has been confirmed several times and work is in progress attempting to isolate the factor responsible. Some investigators think it may be a virus. This does not eliminate either the hereditary or endocrine factors, but inserts an unknown of extreme importance.

While these studies of atypical growths of the mammary glands in response to estrogens were in progress, the whole question of the part played by estrogens in mammary growth has been reopened (27). It was found that estrogen injected into animals after hypophysectomy induced very little if any mammary growth (28, 29), even though the vagina and uterus grew normally in response to the injected hormone. This led to the proposal of a pituitary mammogenic hormone, in addition to prolactin, and the theory that injected estrogen stimulated the pituitary to secrete this growth hormone (27).

Then it was shown that estrogen applied locally to the skin over the mammary glands in such very small doses that it did not give a systemic reaction in vagina and uterus, would induce local growth of one mammary gland and leave the adjacent ones quiescent. This has been done both in mice and monkeys (30, 31, 32). Trials are being made clinically by local application to induce mammary growth and to remedy unilateral differences in mammary development.

These latter experiments would seem to make it necessary to modify the hypothesis concerning a mammogenic hormone from the pituitary gland and emphasize instead the need of pituitary secretion for some sustaining function prerequisite to mammary growth under estrogenic stimulation.

It seems clear from experiments with mammary cancer in mice that genetic determination plays a part; that stimulation by estrogenic hormone is necessary; that some pituitary factor plays the essential supportive rôle; and that something transmitted in the mother's milk may be extremely important.

Again in connection with the growth of the mammary glands it should be urged that there is more to hormonal participation than mere stimulation of excessive growth. The onset of recognizable cancerous nodules comes at a time when the major part of the glands are involuting. The persistence of the multiple hyperplastic nodules in such an atrophic system and their availability as potential foci of cancer is noteworthy.

4) *Interstitial cell tumors of the testis.* The recent report that castration in cases

of prostatic cancer in man alleviates pain and other symptoms has focused attention again on endocrines as factors in cancer (33). In the above instance androgenic hormones are probably the ones involved. Recently, however, interstitial cell tumors of the testis have appeared in mice of the *A* strain following long continued injections of estrogenic hormones (34).

Early experiments with estrogens in males showed that, if injections were begun at prepuberal ages, growth of the testes and their descent into the scrotum could be inhibited (35). This 'antagonism' of the homone of the opposite sex was then shown to operate partly by depression of pituitary gonadotropic secretion (36). When concentrated estrogens became available, chronic administration was followed by interstitial cell hypertrophy (37). This diffuse hypertrophied tissue often largely replaced the spermatogenic tubules (38). Some of these tumors are striking when seen at autopsy, for they may have a brownish yellow or orange color, and when they metastasize to regional lymph nodes, the color accentuates the lymphatic chain of nodes. Then in mice of the *A* strain there occurred enlargement usually of one testis (occasionally bilateral) to the point where it might be truly designated a tumor (37, 39). Metastases do occur in fairly high incidence. Whether or not retrogression will follow discontinuance of treatment remains to be tested. The animals are usually past the breeding age when the tumor appears. A certain degree of autonomous growth seems assured by survival of tumor tissues through several generations of transplants into strain *A* mice, but only when injections of estrogen are continued in the hosts. This tumor seems to be definitely strain limited.

In the early stages of the experiment secretion of androgen by the testes decreases, as shown by considerable involution of the male accessory glands. Later, however, after the enlarged testicle is definitely tumorous, waves of growth and secretion in the seminal vesicles and prostate indicate secretion of androgenic hormone by the tumor tissue (39).

5) *Adenomatous tumors of the pituitary.* Enlargement of the pituitary gland during pregnancy had been observed, and hypertrophy after treatment with estrogens had been well established by several experimenters. In 1936 there were several reports of large pituitary tumors in both mice and rats after chronic estrogenic treatment (40, 41, 42). They have been obtained frequently since then. The C_{57} strain seems especially susceptible after long-continued estrogenic treatment. Extreme enlargement, usually due to chromophobic cells, causes pressure symptoms and misshaped skulls. Both male and female rats and mice of some strains react. The pituitary adenoma does not metastasize but does grow when transplated if estrogen is injected into the hosts (43). Pituitary tumor cells from rats, when transplanted to other young rats, may lie dormant until the hosts reach old age when the transplants may grow.

6) *Tumors of the suprarenal cortex.* Endocrinologists have pointed for years to suprarenal cortical tumors as responsible for masculinization phenomena in women. Retrogression of symptoms after removal of the tumors seems conclusive evidence. Secretion of androgen by this tissue or development of masculine characteristics in response to excessive amounts of cortical hormone, seem natural inferences. Many times, however, it is difficult to establish the presence of a cortical tumor when masculinization appears. It is sometimes difficult even to establish hypertrophy of the suprarenal cortex in some of these cases. Excretion of 17-ketosteroids is sometimes high, but often falls within the range of excretion of these substances in normal people.

Recent experiments with ovariectomy in mice indicate that under certain condi-

tions these suprarenal tumors may secrete estrogens. Mice were ovariectomized early in life to test again the effect of ovariectomy upon the incidence of mammary cancer, first reported by Loeb. After castration of many months duration, some of these animals showed signs of returning estrous periods. At first it was thought that regeneration of ovarian tissue had occurred, but at autopsy suprarenal tumors were located, and seemed to be the tissue responsible for return of function to the genital tract of the castrated animals (44).

Attention was called to the possible participation of the suprarenal cortex as one endocrine factor in mammary cancer by Cramer who reported 'brown degeneration' involving the inner zone of the cortex and the medulla associated with a high incidence of mammary cancer (45). That it need not always be so associated, however, has been shown by Blaisdell's observations (46). Then in mice of the several inbred strains on various experimental levels of estrogenic treatment, new growth of local areas of the cortex was observed. In some animals this neogenic tissue is merely a segment of part of the cortex. In others, discrete nodules of cortical tissue may develop and sometimes these attain the size and appearance of definitive tumors (47). These have not been proved capable of growth on transplantation.

7) *Osteogenic transformations of bones.* In birds, normally at the time of ovulation, new bone is laid down in the marrow cavities of some of the bones (48). The pneumatic ones are not involved. A similar process goes on to a limited degree in normal mice toward the end of pregnancy. Non-pregnant mice and male mice on chronic estrogenic treatment may develop almost completely solid bones (49). This is an instance of extreme atypical growth. The almost complete replacement of the marrow cavities by osseous tissue can be induced experimentally in birds by injecting estrogenic hormones (50). These experiments have been followed by clinical trials of estrogen in the hope of accelerating repair of fractures especially in obstinate cases.

Osteogenic sarcomas have been described in one strain of albino mice (51). These tumors appeared after three to four months of estrogenic treatment in both males and females, but with a higher incidence in females.

In addition to tumors, some of which may definitely be classed as cancers, other excessive or atypical conditions which have appeared following estrogenic treatment should be included. Cystic ovaries, following excessive or unbalanced pituitary gonadotropic stimulation or unbalance, are included because of their secretion of estrogen, and the hyperplastic uterine conditions which follow.

1) *Cystic hyperplasia of the endometrium and other atypical uterine responses.* Before the cyclic variations in the endometrium were thoroughly studied, the fully developed secretory phase of the endometrium was classed as a pathological condition and called 'cystic hyperplasia.' Now only extremes of this condition are described by this term. It is possible to carry the secretory phase of the uterus, both as concerns growth of the glands and their secretion, into various abnormal states by prolonging treatment with estrogens (52). This has been done in rats, mice, guinea pigs and rabbits (53). One extreme which occurs occasionally in mice is continued growth of the uterine glands through the muscle layers of the uterus, the tips appearing as distended bubbles under the peritoneum (54). When the ducts are blocked and distended with secretion, considerable cysts may be formed. Other atypical conditions appear in the uterus after long-continued injections of estrogen. If the treatment is begun before puberty, there may be extreme growth of the surface epithelium which may become metaplastic, transforming the entire lining of the uterus

to a stratified epithelium (54). There may be other than hormonal factors concerned with this transformation such as infection, for chronic stimulation with estrogens, without permitting periods of rest or retrogression, lowers the resistance of the uterus to infection (55). The connective tissue of the endometrium also reacts to this type of treatment. Extensive hyalinization of the stroma and the uterine blood vessels is frequently found.

Many of the changes described in experimental animals, such as endometrial moles (55a), are truly atypical and often associated with conditions which may come under the heading of cystic hyperplasia. This involves primarily a hydropic transformation of the uterine stroma. These growths may develop into gelatinous tumors. While they originally form after trauma to the uterus, in the last half of gestation in the rat injections of estrogen seem to increase their development.

2) *Metaplastic and hypertrophic changes in parts of the male accessory organs.* When large doses of estrogen are injected into males some of the accessory sex organs react atypically (56, 57). The muscular and connective tissues of the seminal vesicles hypertrophy (58). Similar but less extensive changes appear in the prostate. The epithelium of part of the urethra and the prostate may become stratified and metaplastic in both mice and monkeys (59). The lobe concerned with coagulation of the ejaculate often becomes distended with sloughed epithelial cells indicative of extreme metaplasia (60). In dogs, injected estrogen induces marked hypertrophy of the prostate (61) which subsides after hormonal stimulation is stopped. The female genital rudiments in the male, such as the uterus masculinus, react strongly. These atypical growths do not occur when testosterone (62) or progesterone (63) are injected simultaneously with an otherwise effective dose of estrogen.

3) *Leukemia.* Abnormal growths which appear in mice on chronic estrogenic treatment include leukemia, usually the lymphatic type (64). This disease in mice is as lethal as in man. In fact, injections with estrogen are the most effective way to obtain a high incidence of leukemia in mice of one inbred strain (65). Certain inbred strains (C_{58}, AKA; McDowell and Furth) are especially susceptible (67).

A discrete tumor mass usually appears in the mediastinum apparently involving the thymus (64). In some animals a generalized systemic leukemia appears later. Just what the action of the hormone is in this particular case remains problematic.

4) *Hypertrophy of the bile ducts.* Extreme hypertrophy has been noted in the bile ducts, both hepatic, cystic and common, in mice of several strains after chronic treatment with estrogen (68). This hypertrophy may be very extreme and may transform a very inconspicuous and semi-transparent normal duct into one twenty or thirty times its size. The smaller ducts in the liver itself are not involved. The mechanism of this hypertrophy is difficult to suggest unless it involves some inability of the liver to detoxify completely excessive amounts of hormone.

5) *Cystic ovaries.* The ovaries are sometimes transformed into tumors by formation of cysts. Early in the study of gonadotropic hormones, attempts to induce ovulation often resulted in cystic ovaries of the follicular type (69). Corpus luteum cysts occur spontaneously in some animals but have not been produced experimentally. Unbalanced anterior pituitary gonadotropic stimulation seems to be involved.

As experimentally produced, more follicles than would otherwise reach this stage of development go into preovulation growth (hyperplasia and secretion of liquor folliculi) but fail to ovulate and then become cystic. This condition can be sustained for considerable periods by continued gonadotropic stimulation, but regression usually sets in promptly after cessation of hormone treatment. A similar ovarian condition occurs in rats in surgical parabiotic union (7, 69). Also in rats pituitary func-

tion can be modified by implants of testes from litter mates at birth so that ovulation is inhibited and the ovaries become cystic (72). Atypical conditions appear in the uterus similar to those induced experimentally by long-continued estrogenic treatment. That a severe experimentally induced cystic condition may be maintained for a considerable time without permanent damage to the ovaries has been demonstrated in monkeys where normal mating, gestation and lactation followed cessation of gonadotropic stimulation (73). These experiments go far toward placing this type of cystic ovaries under the heading of primary pituitary dysfunction.

DISCUSSION

The rationale underlying the experiments with estrogens which seek to induce tumors depends upon the well-established action of estrogenic hormones in inducing growth of the female genital organs. This action is most apparent when compared to the atretic condition of the genital organs of the castrate. Waves of growth can be induced after ovariectomy which are quite comparable to those in normal animals during cyclic function in non-pregnant animals (53). In the normal animals these waves of growth are followed by periods of retrogression. In experiments seeking to produce tumors, the experimenter attempts to keep the organs or tissues growing continuously by a prolonged bombardment with growth stimulating hormone and without permitting periods of rest or retrogression.

Autonomous growth of the experimental cancers listed has been demonstrated by transplantation. Some of the other tumors, while not malignant, attain considerable size. Some of the latter grow when transplanted, only if the hosts are injected with estrogen. Other atypical growths are reversible and retrogress without leaving damage when the hormone is withdrawn.

If carcinogens are defined broadly as agents whose application is followed by cancer, estrogens should be included, especially when doses are massive and treatment is chronic. It should be emphasized, however, that suceptibility and resistance of various species and inbred strains play dominant rôles in both spontaneous and induced tumors.

Comparison of the action of estrogens and other carcinogens establishes some similarities but many differences. For instance, the former occur as natural constituents of the body; the latter must still be regarded as foreign chemicals. The carcinogens, benzpyrene or dibenzanthracene, have usually been identified biologically by their capacity to induce tumors at the sites of application. However, tumors of the lung and of the mammary glands have been ascribed to action of carcinogens applied remotely. Long continued treatment is usually necessary with estrogens for tumorigenesis. A single application of carcinogen may be effective. The hormone usually affects certain reactive organs or tissues far from the site of injection. Exceptions must be made in both cases, however. Sometimes it seems that the estrogen acts directly upon sensitive tissues, at others, probably through the intermediation of another endocrine gland. For instance, the interstitial cell tumors of the testes in mice of strain A, which appear after estrogenic treatment, and where it seems probable that the pituitary is involved (74).

The logic which led to the use of estrogenic hormones in attempts to produce tumors of the female genital organs was that since moderate doses over short periods resulted in estrous (and intermenstrual) growth, excessive and atypical conditions might be induced by larger doses and long-continued treatment. In many cases, however, the growth reaction cannot be sustained in this way for long periods of time. The genital organs show signs of damage such as hyalinization or sclerosis, and de-

cided involution may occur in spite of continuation of the hormone. The tissues seem 'exhausted' from too much hormonal stimulation. For instance, experimental cancer of the cervix uteri in mice appears only after the animals survive 10 or 12 months of estrogenic treatment and frequently vaginal smears or sections show that an estrous condition was not sustained toward the end of this period (16). Therefore, it is probable that something more than excessive growth is operating. Involution of the tissues around the lesion may have reached a stage comparable to that of old age changes in normal animals. The same may apply to mammary cancer in mice following estrogenic treatment for, with the exception of multiple hyperplastic nodules, the mammary gland may be atrophic (22). This might have been expected when it is recalled that the highest incidence of cancer is associated with atrophic organs in aging people.

In connection with estrogenic hormones injected for purposes of carcinogenesis, damage to the protective mechanisms of the body must be considered. Destruction of estrogen in the liver, or its combination or conjugation there to render large doses less effective, has been demonstrated experimentally by perfusion of estrogen through the liver (75) and by incubation of estrogen with liver tissues (76). Damage to the liver in experimental animals may reduce the time before appearance of certain types of tumors (77).

The matter of lowered resistance must also be considered in relation to infective agents or viruses, especially the latter, which may enter into the final result. So the hormonal stimulation may be only one of several factors. The fact remains, however, that hormonal treatment at high levels for long periods is followed by appearance of atypical growths, including tumors and cancers in some experimental animals in such high incidences that the endocrine stimulation may play a considerable, rather than an incidental, rôle.

SUMMARY

1. Long-continued treatment with high doses of estrogenic hormone is followed by appearance of tumors and cancers in experimental animals.

2. Genital organs are involved primarily, but non-genital tissues are also affected.

3. The uterus may develop cancer of the cervix (in mice) or fibromyomas of the cornua (in guinea pigs).

4. The mammary glands are especially susceptible to cancer. In certain inbred strains of mice low tumor-incidence among females is increased by estrogenic treatment. Males of strains whose females are susceptible to spontaneous mammary cancer may have the disease after injections of estrogen.

5. The testes of mice respond to this estrogenic treatment by hypertrophy of the interstitial cells. In the A strain unilateral nodules may become large tumors. These are transplantable into other mice of this strain if estrogen is injected into the hosts. The tumor tissue may secrete androgenic hormone.

6. Pituitary hypertrophy and chromophobic tumors of considerable size can be induced in high incidences in mice of certain strains and in rats.

7. The suprarenal cortex may develop tumors after long estrogenic treatment in mice. Mice ovariectomized at early ages resumed estrous cycles late in life. Cortical tumors found at autopsy are considered responsible for the secretion of estrogen in these cases.

8. Growth of new bone in the marrow cavities of mice and several species of birds may be induced by estrogenic hormone. Osteogenic tumors have been reported in albino mice.

9. Several hyperplastic or hypertrophic conditions, definitely atypical, have fol-

lowed excessive estrogenic stimulation. These include cystic hyperplasia of the uterus, metaplastic changes of male accessory organs, leukemia (lymphatic), and hypertrophy of the bile ducts.

10. Cystic ovaries may be induced by unbalanced pituitary stimulation. In these cases hypertrophic uterine conditions are induced by endogenous estrogen.

11. From these experiments endocrine secretions appear as important factors in the genesis of some atypical growths, including certain tumors and cancers.

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ENDOCRINE EFFECTS OF CERTAIN DYSONTOGENETIC TUMORS OF THE OVARY

EMIL NOVAK

AN INTERESTING BY-PRODUCT of the endocrinological investigations of recent years has been the demonstration of the fact that certain tumors of endocrine glands retain the functional properties of their constituent cells, and that they may therefore produce important and even profound biological effects. This is a far cry from the earlier concept of tumors as purely parasitic structures, made up of cells with exaggerated growth capacity, but not participating in the functional activities of normal body cells. Even in tumors of non-endocrine structures, the constituent cells often retain considerable degrees of functional activity, good examples being the production of mucin by certain carcinomas of the intestinal canal, and of bile by certain cancers of the liver.

The functioning type of tumor, however, is best exemplified in the domain of the endocrine glands, perhaps because the resulting hormonal disturbances are reflected in phenomena affecting distant organs and tissues. An eosinophilic adenoma of the pituitary gland, for instance, produces the striking body changes of gigantism or acromegaly, though the tumor itself be so small as to produce little or no local effect. Many other examples of such endocrine tumors, producing profound distant effects, may now be adduced, such as basophilic and chromophobic pituitary adenoma, certain tumors of the adrenal, the pancreas and the gonads, both male and female.

Histogenesis and Types of Functioning Ovarian Tumors

Gynecologists have been particularly interested in a group of tumors originating in the ovary which are capable of producing striking effects upon the sex characteristics of the individual. Most of these tumors have been known to pathologists for many years, though under other names than are applied to them now. The newer designations are based upon our recently acquired knowledge as to their histogenesis, and the parallel development of our knowledge of the nature and function of the sex hormones. The most important members of this group of ovarian neoplasms arise from cells dating back to the early stages of ovarian embryology, so that they are properly spoken of as of dysontogenetic type. As a matter of fact, no intelligent concept of the histogenesis, the pathology or the functional properties of these tumors is possible without some knowlege of the early development of the ovary, a subject concerning which our knowledge is still very incomplete. What is said on this point, therefore, must be evaluated with due regard to these deficiencies in our knowledge, although these will scarcely have any important bearing upon the working theory which has been evolved as to the functional activity of the tumors in question.

The sex gland anlage is formed by an accumulation of cells just beneath the celomic epithelium on the anterior or ventral surface of the Wolffian body. In the earliest phase of development, the indifferent phase, it is impossible by histological examination to determine whether the sex gland is to develop into an ovary or a testis. It is of interest to note that in this indifferent phase certain cells may be disassociated from the germinal stream, so to speak, and that they may in later life give rise to a

tumor type known as dysgerminoma. As might be expected, such tumors possess no endocrine function. Also as might be expected, they may occur in gonads which have differentiated into either ovaries or testes, although in the latter location they are more commonly spoken of as seminomas.

The first evidence of sex differentiation is to be seen in the development of the so-called sex cords or medullary tubules, which soon converge in rather zig-zag fashion toward the hilum of the gland. In the case of the gonad destined to develop as a testis, these cords become canalized, and later constitute the seminiferous tubules. Through junction with Wolffian structures, the whole testicular structure is soon laid down, the Wolffian duct itself becoming the vas deferens.

Even when the gonad is to become an ovary, the same preliminary sex cord differentiation is noted, but it soon retrogresses, while further ovarian differentiation proceeds over the fossil remains of this earlier 'testicular' scaffolding. As a matter of fact, certain cells of the preliminary testicular phase, with male-directed potentialities, may persist in the medulla of the ovary, in the region of the so-called rete ovarii. According to Robert Meyer (1) whose viewpoint has been quite generally accepted, it is from such male-directed cells that there may later develop in the ovary tumors which histologically suggest various phases, differentiated or undifferentiated, of testicular development, and which functionally are characterized by masculinization effects. To this tumor group is applied the designation of arrhenoblastoma.

To proceed with the further development of the ovary, the retrogression of the sex cords is followed, with overlapping, by a second wave of differentiation, with the formation of cell columns which now are to be considered as definitely female. We need not here discuss the still controversial question of whether the cell cords or columns are the results of invagination from the germinal epithelium or whether they are formed by differentiation from the ovarian mesenchyme in situ. Suffice it to say that the evidence of recent years indicates the probable correctness of the latter view, and that the ovarian mesenchyme is to be looked upon as the parent tissue of both granulosal and thecal cells. This is a point of some importance in our understanding of the feminizing group of tumors which have their source from cells segregated in this early phase of development, when the gonad is already distinctly stamped as female. Such tumors may consist of epithelial (granulosal) or connective tissue (thecal) elements, or a mixture of the two, although in either event they produce estrogenic or feminizing effects. On the basis of their granulosal or thecal cell composition, we may thus distinguish the two chief types of feminizing tumors as the granulosa cell tumors and the thecomas.

The further differentiation of the follicular apparatus is accomplished by the grouping of epithelial cells about the germ cells or oögonia, with the formation of the primitive follicles. In this process clumps of redundant granulosal cells may be left over as granulosal rests, and these are sometimes seen in the ovaries of children and, less frequently, of adults. Meyer looks upon these as the origin of granulosa cell growths. While this is possible, it would seem more likely, in view of what has been said above, that the origin is more frequently to be traced to a progranulosal and prothecal phase, i.e. to the parent ovarian mesenchyme.

This bald and sketchy outline will at least serve to indicate the present day views as to the origin of the chief masculinizing and feminizing tumors of the ovary. Our knowledge of sex differentiation is still too incomplete to justify any arbitrariness of viewpoint, and it is quite certain that future progress along this line will necessitate modification or correction of present day views. Even now, for example, there is reason to doubt the correctness of the explanation of arrhenoblastoma given in a pre-

ceding paragraph, since biologists are now stressing the probable importance of the cortical and medullary sex determiners or organizers. It is of interest to note the almost perfect similarity of the endocrine effects produced by certain tumors of the adrenal cortex on the one hand and certain medullary tumors of the ovary (arrhenoblastoma) on the other. It is difficult to escape the belief that an important factor in this relation is the intimate embryologic relationship of the two tissues concerned.

Histological Variations of Functioning Ovarian Tumors

The endocrinologist will probably be far less interested than the gynecologist in the pathology and histology of these special tumor types. For this reason, therefore, the present paper concerns itself chiefly and briefly with the clinical and endocrinological characteristics of these tumors, with only scant discussion of pathology and histology. Even the most expert pathologist might be at a loss in interpreting the various microscopic patterns produced by these tumors unless he has some familiarity with the early embryology of the gonads. In the case of the granulosa cell tumors there are all sorts of variations in pattern, but the diagnosis can ordinarily be made quite readily on the basis of the granulosa cell morphology and the growth characteristics of the constituent cells, which are those exhibited by granulosal tissue.

The problem is somewhat more difficult with arrhenoblastoma, which really embraces a whole series of possible histological types, representing various degrees of approach, usually very imperfect and abortive, toward testicular tissue. In the most highly differentiated variety, the so-called testicular adenoma, the reproduction of testicular tissue is very obvious, although spermatogenesis has never been observed. At the other extreme, one sees highly undifferentiated varieties which for the most part resemble sarcoma, the only clue to their real nature being an arrangement of the cells, in at least some portions of the tumor, into columns reminiscent of the sex cords seen in early phases of testicular embryology. In the intermediate varieties all gradation between these extremes are encountered, most of the intermediate group showing a greater or less number of tubules and some showing collections of cells which resemble in every way the interstitial cells of Leydig.

In a small proportion of granulosa cell tumors parts of the tumor show a luteinlike transformation of the granulosa cells (folliculome lipidique of Lecéne), and occasionally this luteinization involves the entire growth, producing the so-called l u t e o m a . There is good reason to believe that most luteomas arise in this fashion. In a few instances, such as those reported by Novak (2) Te Linde and Henriksen (3), and Arnold, Koerner and Mathias (4), there was an actual production of progesterone, as shown by the decidual or predecidual transformation of the endometrium. More often, however, the effects of tumors showing luteinization of granulosa have appeared to be estrogenic, suggesting that the cell changes must perhaps be interpreted as merely pseudolutein in character, the cell metamorphosis involving the morphology but not the function of the cell. When progesterone effects are actually produced there would seem to be little doubt of the intervention of the same hypophyseogonadal reaction which operates during the normal cycle, although one can only speculate on this point.

Much confusion has risen because a small group of luteomas have been reported to produce masculine rather than feminine effects, but Schiller (5) is probably correct in his statement that with few exceptions such tumors have been of adrenal rather than lutein origin. This whole group of neoplasms, small though it is, is still a very confusing one, and Rottino and McGrath (6) have suggested for it the designation of m a s c u l i n o v o b l a s t o m a .

In this feminizing group one must of course place the thecoma. As already mentioned, this must be looked upon as an exceedingly close relative or mere variant of the granulosa cell tumors. Like the latter, it may show various degrees of luteinization of its constituent cells.

The masculinizing group embraces the arrhenoblastomas and the even more rare adrenal tumors of the ovary, with the doubtful inclusion of a few luteomas, as also mentioned above. The very close embryological relation of the ovarian medulla and the adrenal cortex may explain the identity of the masculinization syndromes produced by these tumors, and certainly cannot be pushed aside altogether by the prevalent concept that the effects of arrhenoblastoma are due simply to an origin from male-directed cells included in the ovary, and those of adrenal cortical tumors due to some unknown cortico-gonadal interrelationship.

ENDOCRINE EFFECTS OF TUMORS

Feminizing group. The feminizing group, embracing the various forms of granulosa cell tumor and thecoma, have been shown to produce estrogenic substance. For example, injections of tumor extract or implantation of tumor tissue into castrated animals brings about definite estrous phenomena (7). The clinical effects produced by these neoplasms are therefore those of hyperestrogenism, aside from such symptoms as might be brought about by any of the ordinary types of ovarian tumors. The effects of hyperestrogenism, however, vary greatly, depending on the life phase of the individual.

In infancy and early childhood one would expect that persistent hyperestrogenism would evoke the phenomena of precocious puberty, inasmuch as the unfolding of secondary sex changes at the normal puberal epoch is unquestionably due to the inauguration of ovarian estrogenic function at that life phase. This is exactly what occurs, and the causal relation of the tumor to the precocious puberty is demonstrated by the prompt regression of the precocious manifestations after removal of the tumor. Indeed, in at least one recorded case, later recurrence of the tumor in the opposite ovary again evoked precocious pubertal phenomena, with again their regression after removal of the second tumor.

When these so-called feminizing tumors develop during reproductive life, as the largest proportion do, their endocrine effects would not be expected to be as sharply defined as when they are, so to speak, projected against the estrogen-bare background of the immature child. In the woman of menstrual life, considerable estrogen is normally already present, and the secondary sex characteristics are fully developed, so that the endocrine effect of the tumor is purely quantitative, and not materially different from the functional hyperestrogenism which is so frequently encountered, chiefly in association with functional bleeding, but at times with amenorrhea. In other words, menstruation may be either excessive or normal in amount, or it may be in abeyance for long periods, while other endocrine phenomena are lacking.

Finally, in the not infrequent cases in which granulosa cell tumors or thecomas have occurred in women well beyond the menopause, one must interpret the clinical phenomena on the basis of the possible effects of excessive estrogen upon the recipient tissues of that particular life phase. Such tissues as the breast have apparently lost their sensitivity to estrogen, and certainly no effect would be expected on hair distribution. On the other hand, the uterus is capable of responding to estrogen long after the menopause, or for that matter, to the very end of even long life. The characteristic effect of tumors of this variety upon the postmenopausal woman are the appearance of bleeding of a more or less periodic pseudomenstrual type, together with a greater or less degree of growth effect upon the musculature of the uterus,

which may be restored to the size of the organ during reproductive life. The menstruation-like bleeding is characteristically of the estrogen-induced variety, and the endometrium usually exhibits the picture of hyperplasia. Regression of the uterine changes, with cessation of the bleeding, may be expected to follow removal of the tumor. It is of interest to note that in a number of recorded instances a second menopause has occurred, with typical vasomotor symptoms (2, 8, 9).

Tumors of the feminizing group are by no means rare, and something like 100 cases have been encountered in the material of our Laboratory of Gynecological Pathology at the Johns Hopkins Hospital. They constitute about 10 per cent of all primary solid carcinomas of the ovary. Since some tumors of this group have been cured by conservative operations, there has been a tendency on the part of some authors not to include this neoplastic group in the group of ovarian carcinomas. There can be no question, however, as to their malignant potentialities, and in many instances, the tumors have run a highly malignant course, with recurrence after even radical operation, sometimes very soon, sometimes not for many years.

Masculinizing group. As regards the endocrine effects of the masculinizing group of tumors, these may be divided into a first phase characterized by defeminization rather than actual masculinization, followed by a second series of manifestations frankly of masculinizing character. In the typical case, amenorrhea is almost always the first symptom, followed by flattening of the breast, and, though it is much less constantly noted, loss of the rounded contour characteristic of the female. All of these changes subtract something from the feminine, but cannot be considered positively masculinizing, as are the later manifestations, including especially hirsutism, deepening of the voice, and hypertrophy of the clitoris.

Removal of the tumor is followed by prompt disappearance of the defeminizing manifestations. Menstruation reappears ordinarily about one month later, and the breasts resume their former size within a comparatively short time. The masculinizing symptoms, however, regress far more slowly. The abdominal hair gradually disappears, but often not for many months and not always completely. Even slower is apt to be the regression of the clitoris, and when hypertrophy has been marked, amputation of the organ may be necessary.

The symptoms of the rare cortical adrenal tumors of the ovary are practically indistinguishable from those above described, with a similar tendency toward disappearance after removal of the tumor.

The incidence of arrhenoblastoma is far less than that of granulosa cell tumors or thecoma. A review of the literature (10) in 1938 yielded only 55 cases, although a small number have been reported since then, and many cases have undoubtedly escaped recognition.

Struma ovarii. One other variety of ovarian tumor possessing endocrine capacities is the so-called struma ovarii, or thyroid tissue tumor. In a number of recorded instances, the thyroid tissue in the tumor has become hyperplastic, producing symptoms of hyperthyroidism. The origin of these tumors is believed to be teratomatous, the thyroid tissue blotting out other alien elements so that a large part of the tumor, or perhaps even its entirety, is made up of genuine thyroid tissue, on the basis of both histology and chemical content. A review of the literature on this neoplastic variety is available in the recent paper by Emge (11).

SUMMARY

Certain tumors of the ovary are made up of cells believed to date from the early stages of gonadogenesis. When the cells are disassociated from the developmental stream in the early undifferentiated phase of gonadal embryology, the resulting tu-

mors have no endocrine potentialities, and they may occur in either the ovary (dysgerminoma) or testis (seminoma). When tumors arise from certain elements persisting in the ovary and possess originally masculine potentialities, masculinizing tumors are produced (arrhenoblastoma), although we cannot be sure that other still poorly understood factors in sex differentiation may not play an important part in bringing about the sex character changes associated with such tumors. Finally, tumors arising from cells of the female mesenchyme, and assuming the form of either granulosa cell tumor or thecoma possess definite feminizing effects dependent on their estrogenic function, and these manifestations depend much on the age at which such tumors occur. The clinical aspects of these tumors are briefly discussed in this paper.

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TRANSFORMATION OF MALES TO INTERSEXES BY ESTROGEN PASSED FROM BLOOD OF RING DOVES TO THEIR OVARIAN EGGS

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THE FIRST PURPOSE of the present study was to learn whether female ring doves in reproduction can or do dispose of a small extra quantity of blood estrogen by placing it in their growing ova. It was thought possible to obtain a decision on this point by injecting estrogen (in oil) intramuscularly into doves known to be in the process of maturing an ovum, by hatching offspring from ova thus exposed to estrogen, and by noting thereafter the presence or absence of characteristic modifications in the testes and gonoducts of male offspring from such eggs. It seemed probable that at least a part of the excess of females, and some grades of male intersexuality, which were earlier observed by Riddle (1–3) in the offspring of doves subjected to 'crowded reproduction,' were in fact induced by excess estrogen in the eggs which produced these intersexes. The evidence for this probability has been increased during the past 7 years by several investigators (4–11) who have shown that the injection of estrogens into hens' eggs during early days of incubation produces changes in the form (flattening) and structure (ovotestis) of the left testis along with hypertrophy or persistence of left oviducts. These studies further showed that the amount of sexual transformation was roughly proportional to dosage and that, in order to be effective, the treatment must begin before the disappearance (at 12th day of incubation) of the incipient ovarian cortex which is 'normally' found on the left testis of the early chick embryo. Therefore, if estrogen can be placed within the ovarian egg by the mother bird it should then probably produce a maximal effect.

Evanescent traces of ovarian cortex on the left testis of the chick embryo were observed long ago by Laulanié (12) and more recently a similar condition has been found in several other species of birds (13–16). It was necessary therefore to learn, first of all, whether such an ovarian cortex is present also on testes of ring doves. A thorough study of the question of the time at which the maximum of cortical development occurs in both doves and pigeons is in progress (Lahr and Riddle). The present study has shown that traces of ovarian cortical tissue are present on the left testes of untreated dove embryos at hatching (14–15 days incubation) in a high proportion of cases, and that such traces are very rare in post-hatching stages. These data provide a suitable control for the experimental part of the present study which is concerned chiefly with modifications (by estrogen) of the testes and gonoducts at hatching and during the first few weeks thereafter.

Some years ago estrogens were fed to laying hens by Schouppé (17), Lang (18) and Ökland (19) who recorded the sex ratio obtained from eggs laid during the period of feeding. Unlike results were reported by these observers, but the activity of the orally administered estrogen is questionable and histological study of the gonads seems to have been entirely omitted. Apparently those studies require no further mention here.

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MATERIALS AND METHODS

Most of the females treated in this study were blond ring doves (*Streptopelia decaocta*, syn. *risoria*) whose mates were white males. Color of offspring is here sex-linked, the males being of brown or blond color and the females white. Toward the end of the study a few offspring from pairs of blond birds were used since, with resort to sectioning of the gonads, no case of extreme modification of the gonads had been found such as would leave the genetic sex of an individual in doubt.

Female ring doves lay two eggs in a clutch. The first egg of such a pair precedes the second by about 40 hours and can be palpated within the oviduct at least 33 or 34 hours before it is to be laid. 'First' eggs are laid at or near 5:00 P.M. and 'second' eggs are ovulated about 2 hours later (about 7:00 P.M.). Since these time relationships are essentially regular, and the presence of a soft shell or a well-calcified shell indicates the day on which the egg is to be laid, a substance may be administered at any desired number of hours from 1 to 34 before the second ovum completes its growth and breaks from the ovary. When substances soluble in fat are thus injected these will have little chance to become incorporated into the first egg, which is then already in the oviduct where it acquires only albumen and a shell; but sterols and other lipids may readily enter the rapidly growing yolk of the second egg which is still in the ovary. All males derived from these second, or ovarian, eggs, and several males derived from first, or oviducal, eggs were studied with a view to learning whether the treatment had modified the development of their testes and gonoducts.

Two crystalline sterols,[1] estradiol benzoate and dehydroandrosterone, were utilized. The hormone was dissolved in sesame oil in such concentration that no more than 0.2 cc. had to be given. All treatments were by single intramuscular injection; this was given never less than 26 hours before the second egg would complete its growth in the ovary and mostly at 30 or 34 hours prior to its ovulation.

Experimental birds were autopsied at various ages (computed from the date of hatching) from 0 days to 9.2 months and a careful examination of the reproductive system was then made. In some cases autopsy followed death by disease but most of the birds were killed while apparently healthy. On gross inspection the left testis of a few of these males was apparently modified in the female direction, the left gonad being larger and flattened. These looked like ovotestes or ovaries; histological study, however, provided the information presented here. A number of these males also had left oviducts, or remains of either right or left oviducts (or both), and these were usually preserved and sectioned. The tabulated data on Müllerian ducts (at hatching and thereafter) relate to macroscopically observable structures which assist and affect the determination of the sexual status of the individual.

Normal Occurrence of Ovarian Cortex on Testis

This part of the study involved only birds autopsied at hatching or within 4 weeks thereafter. The summary given in table 1 shows that the left testis of the dove usually has, or retains, at least some traces of ovarian cortex at hatching; serial sections of the left gonads of 13 of 17 males examined at hatching disclosed such traces. The extreme of cortical development observed in a control bird is illustrated in figures 1 and 2. Apparently a trace of this cortex may persist in rare cases for as much as 2 weeks after hatching. The one case in which a trace of cortex was present in a bird killed 22 days after hatching is neither a typical treated case nor a typical control, since it was hatched from an egg produced by a mother which had been injected 13

[1] The hormones were kindly supplied by Dr. Erwin Schwenk of the Schering Corp., Bloomfield, N. J.

days earlier with estradiol benzoate and this bird was from the next following clutch laid.

Right testes from 11 control males, 8 at the hatching stage, were sectioned serially and studied; right testes from 3 atypical control, and 12 from treated birds (table 2), were studied similarly. In none of these 26 cases was any trace of cortex found on a right testis.

Experimental Modification of Sex

Results with dehydroandrosterone. In another study (Riddle and Lahr, unpublished) dehydroandrosterone (1.5 mg. daily for 7 days) was found to have some but

TABLE 1. DATA FOR CONTROL BIRDS AND FOR OTHERS HATCHED FROM EGGS PRESENT IN OVIDUCT AT TIME OF INJECTION OF ESTRADIOL BENZOATE

Dose, mg.	Time Between Hatching and Autopsy, days	Number of		Number of Left Testes Having Ovarian Cortex and Remarks on Cortex
		Cases	Right testes studied	
		Controls		
0	0	17	8	13
0	4–7	2	1	0
0	10–11	5	1	0
0	14	3	1	1 Minute traces only
0	18–22	4	0	0
Totals		31	11	14
		Atypical control and atypical test		
?	7	1	1	1?[1] No follicles developed
?	22	2	2	1[1] Small trace only
		From 'first' eggs of estradiol-treated clutches[2]		
1.0	1	1	0	1? Dead; poorly preserved
0.5	7	1	1	1? No follicles developed
0.5	14–16	6	6	3? Slight in all; 1 very doubtful
0.5	22	1	1	0
0.5	28	1	1	1 Few follicles present
Totals		10	9	3–6

[1] Hatched from egg laid by female injected with estradiol benzoate 19 (or 13) days previously.
[2] Egg in oviduct 24–32 hours after injection.

rather slight ability to stimulate the immature oviducts of doves aged 1.5 to 3.0 months; this ability is less than 1 per cent of that of estradiol benzoate. Others have noted the probable estrogenic action of this compound and Gaarenstroom (20) demonstrated its estrogenic action in chicks. Present results on eight males hatched from ovarian eggs treated with dehydroandrosterone are summarized in table 2. In one of the two birds of this series killed at hatching the left testis had a very prominent cortex (fig. 3) which exceeded that of the most extreme control (fig. 1, 2) in our series; in the other bird killed at hatching after dehydroandrosterone, however, no trace of cortex of the left testis was found. Again, a male killed 30 days after hatching had a trace of cortex with a few developed follicles on the left testis. Neither of these two instances is duplicated in our more extensive group of control birds. Our tests with dehydroandrosterone involve too few cases, however, to prove an estrogenic effect on the testis; they do nevertheless clearly show, as is to be expected from its very

low estrogenic potency, that 2.0 or 3.0 mg. of this hormone is less effective than one-third or one-fourth of those quantities of estradiol benzoate in developing and maintaining Müllerian ducts and ovarian cortex on left testes of dove embryos.

Results with estradiol benzoate. The results from a series of 17 males hatched from second eggs in clutches treated with estradiol benzoate are summarized in table 2. Twelve of 13 males killed between the ages of 6 and 26 days had a left ovotestis. This result is highly significant. The apparent normalcy of the testes of two males allowed to live 7.6 months (retained Müllerian rests) and 9.2 months suggests that the modification of cortical development by this treatment tended to disappear in the adult sex life of these birds.

TABLE 2. SUMMARY OF RESULTS OBTAINED ON MALES FROM OVARIAN EGGS OF MOTHERS TREATED WITH ESTRADIOL BENZOATE AND DEHYDROANDROSTERONE

Dose, mg.	Time Between Hatching and Autopsy, days	Number of Cases	Number with: Right oviduct only (rests)	Number with: Left oviduct only (rests)	Number with: Oviduct (rests) both sides	Number of Left Testes Having Ovarian Cortex and Remarks on Cortex
Dehydroandrosterone treatment of mother						
3.0	0	2	0	0	0	1, Cortex exceeded any of 17 control
2.0	14	1	0	0	0	0
2.0	28–35	5	0	0	0	1, Few follicles present at 30 days
Totals		8	0	0	0	2
Estradiol benzoate treatment of mother						
1.0	21–22	4	1	1	2	4, Extensive in 3; slight in 1
1.0	7.6 mo.	1	0	0	1	0
0.5	6	1	0	0	0	1, Present without follicular development
0.5	14	3	0	0	2	3, Significant development in all[1]
0.5	21–24	4	0	1	2	3, Follicles in all (3) cases
0.5	26	1	0	1	0	1, Numerous follicles
0.5	33–35	2	0	0	0	0
0.5	9.2 mo.	1	0	0	0	0[2] Apparently normal male
Totals		17	1	3	7	12

[1] Follicles were developed significantly beyond that found on a (single) 14-day control in all 3 birds.
[2] This large testis was apparently perfectly normal but complete sectioning was not done to prove it.

In most of the 12 cases cited in table 2 the degree of ovarian cortical development on the left testis was quite striking as compared with any comparable controls (fig. 8 to 11). In most of these 12 birds follicles were relatively numerous and in general quite like those in the ovaries of control females at comparable ages (fig. 11). There can be little doubt that these ovotestes owe both their existence (at this late stage) and also their relatively high degree of development to the experimental treatment. This statement is not really contradicted by the fact that very poor cortical development on a left testis was noted in one case at 14 days.

In unpublished studies we have observed that estradiol benzoate sometimes causes a 24-hour delay in the ovulation of the second egg and we here note that four of the males of our series, all significantly affected, were hatched from such eggs. Indeed, a single dose of 1.0 mg. of estradiol benzoate sometimes altogether prevents the normal ovulation of the egg.

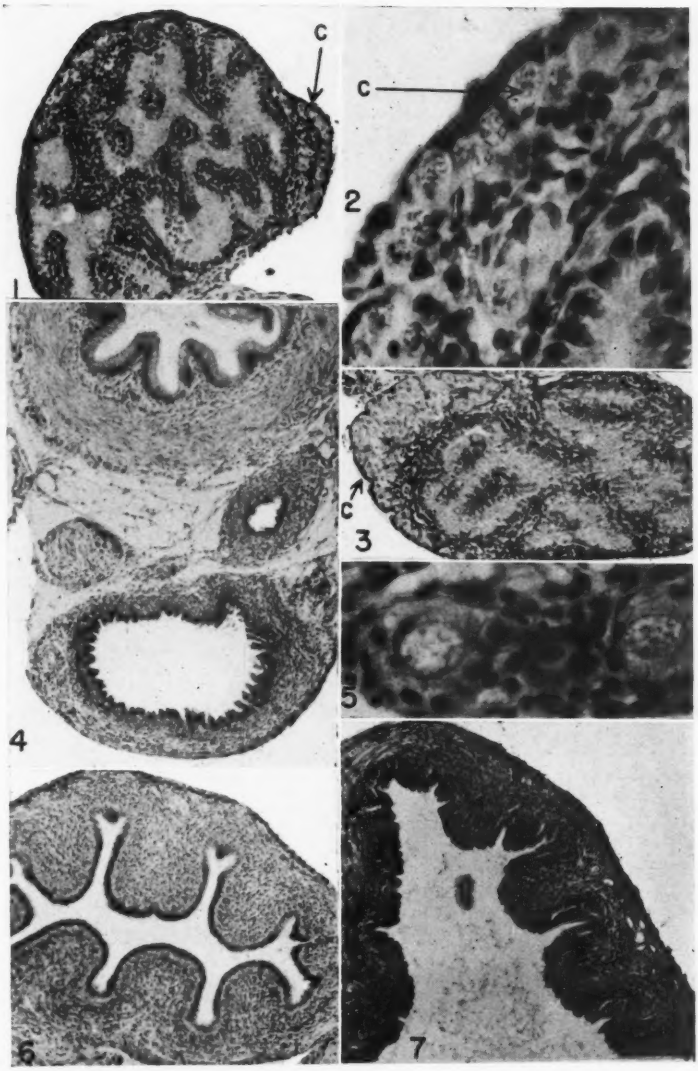

Fig. 1. Left testis of untreated ring dove at hatching, with the maximum of cortex (outside albuginea) observed in control birds. × 126.

Fig. 2. Higher magnification of the cortex shown in figure 1. × 562.

Fig. 3. Extensive ovarian cortex in left gonad of a genetic male ring dove at hatching. From egg treated with 3.0 mg. of dehydroandrosterone. × 126.

Fig. 4. Cross section of ureter, vas deferens and müllerian 'rest' from genetic male dove 21 days after hatching from egg treated with 1.0 mg. of estradiol benzoate. × 63.

Fig. 5. Two follicles, at 16 days after hatching, in trace of cortex on left testis of a genetic male dove hatched from a first egg of a clutch treated with 0.5 mg. of estradiol benzoate. × 562.

Fig. 6. Cross section of oviduct from an untreated female ring dove 14 days after hatching. × 63.

Fig. 7. Cross section of müllerian 'rest' from a genetic male dove hatched from an egg treated with 1.0 mg. of estradiol benzoate and killed at 7.6 months. × 63.

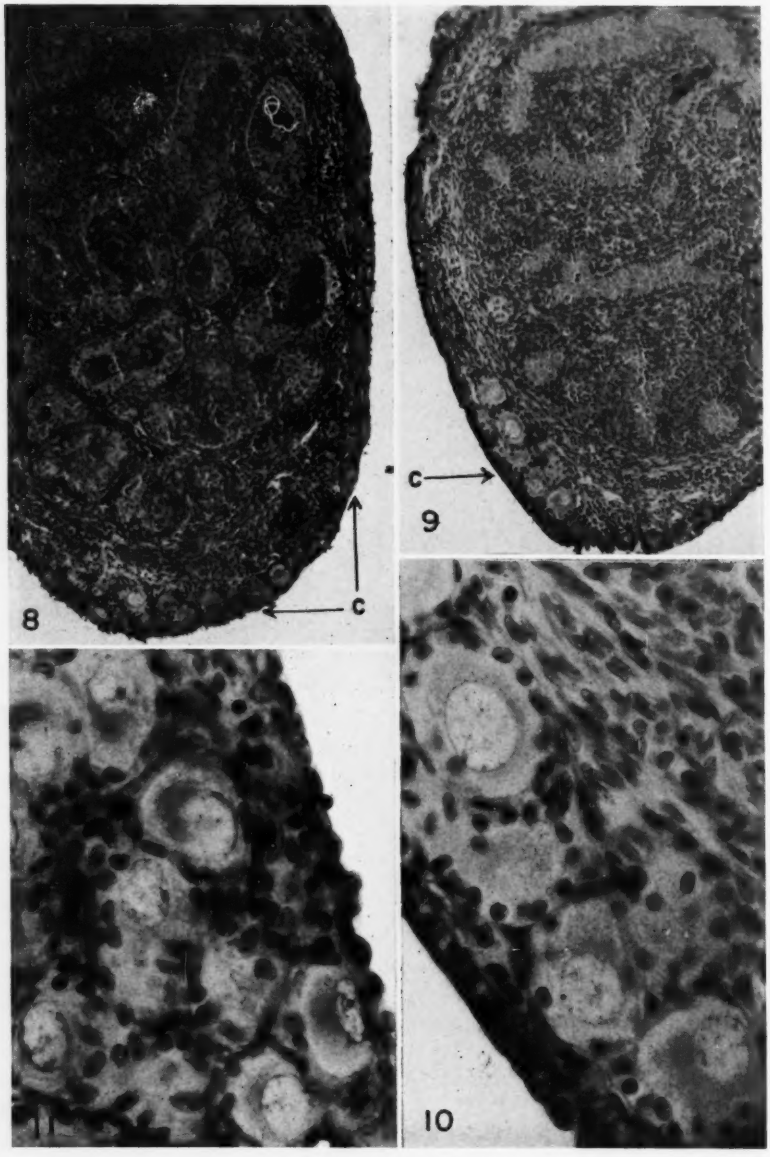

Fig. 8. EXTENSIVE CORTEX WITH NUMEROUS FOLLICLES IN LEFT OVOTESTIS of a genetic male ring dove hatched from egg treated with 0.5 mg. of estradiol benzoate and killed 26 days after hatching. × 126.

Fig. 9. LEFT OVOTESTIS, CORTEX WITH WELLL-DEVELOPED FOLLICLES, of a genetic male dove from egg treated with 1.0 mg. of estradiol benzoate and killed 22 days after hatching. × 126.

Fig. 10. SAME AS FIGURE 9; higher magnification of follicles.

Fig. 11. CORTEX OF OVARY from an untreated female ring dove killed 21 days after hatching. × 562

Oviducts or macroscopically observable remains of right or left oviducts, or of both, occurred in 11 of the total of 17 cases. The special cyst-like oviducal rests, remnants which were observed in 9 of these 11 cases, practically never occur spontaneously in doves of this colony; they were mere fluid-filled, cyst-like structures, more or less spherical and approximately 5 to 10 mm. in diameter, but they were relatively conspicuous on gross inspection. Their location varied from a point near the middle of the kidney to a point just posterior to it. Proof of the oviducal nature of these structures was in all cases based upon histological study (fig. 4). In two cases full-length left oviducts were present. In one of these (fig. 12A) a fluid-filled enlargement formed

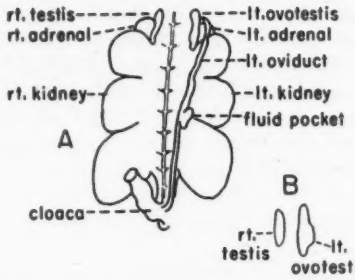

Fig. 12. Organs of genetic males from eggs treated with estradiol benzoate. *A*, Left oviduct with fluid-filled cyst and modified gonads (dose 1.0 mg.) at 22 days after hatching. *B*, Gonads modified by dose of 0.5 mg.; left ovotestis, flattened and enlarged, with prominent cortex at 24 days after hatching.

near the middle of the otherwise rather small duct. The other full length oviduct was found in a bird killed 23 days after hatching and was quite similar in appearance and weight (9 mg.) to the oviduct of a normal female of this age.

One male hatched from a treated egg and killed 7.6 months after hatching had oviducal rests on both right and left sides. A low power photograph of a section of this structure (fig. 7) may be compared with a photograph of a section of oviduct from a normal female (fig. 6) killed 2 weeks after hatching. It appears that the oviduct from the female, 2 weeks old, is slightly better developed than the oviducal remnant found in the male.

The data for 10 males from first eggs, treated with estradiol benzoate while in the oviduct, are shown in table 1. In no case was a cyst-like remnant of an oviduct found at autopsy; and cortical development, although apparently somewhat better than in the uninjected control, was lacking or poor (fig. 5) as compared with that obtained from ovarian eggs. The case which showed cortex with a few follicles at 28 days provides our best single item of evidence that males hatched from eggs already in the oviduct of the mother at the time of injection may be slightly affected by this treatment. The present results therefore suggest that the injected estrogen, being more soluble in fat, is readily carried into the yolk of the maturing ovum but is less readily carried into the egg albumen.

No data are presented here for females although a few birds hatched from treated ovarian eggs were grossly examined, mostly within 3 weeks after hatching, without noting any marked effects; particularly notable is the absence of fluid-filled cysts on the oviducts of the genetic females. The very few specimens of left ovaries studied histologically were structurally normal.

When measured on the quiescent oviducts of very immature doves, 1.5 to 3.0 months old, the estrogenic activity of dehydroandrosterone is surely less than one-

hundredth part of that of an equal quantity of estradiol benzoate. In such juvenile birds a daily dose of 0.1 mg. of estradiol benzoate continues to enlarge the oviduct from a weight of 20 mg. to 1900 mg. during at least a 21-day period (10 tests). Moreover, measurements made on the temporarily quiescent oviducts of mature doves indicate that the total dose of estradiol (0.5 and 1.0 mg.) used in the above tests develops far less estrogenic potency than is developed by a dove while she normally matures a pair (clutch) of eggs. In the latter process Riddle (21) noted that the oviduct of the dove normally enlarges from about 350 to 550 milligrams to 4.000 to 5.500 grams within a period of about 108 hours. In our tests of this item 5 females in reproductive rest were each injected three times with 1.0 mg. of estradiol benzoate (at 112, 72 and 24 hours before autopsy), and their oviducts were then found to have increased from about 365 mg. (range 250–570 mg. in 8 control birds) to 2.223 gm. (range, 1.940–2.385 gm.). In other words, 3 mg. of estradiol benzoate thus administered resulted in less oviducal growth than normally results from estrogen secreted by the bird herself while her ova complete their period of rapid growth. In this connection it should be noted that Dingemanse and Laqueur (22) found that scarcely more than 50 per cent of estradiol benzoate in oil is absorbed within 48 hours after its subcutaneous injection in rats; also that following its injection this substance does not circulate as the benzoate ester but as the free hormone.

COMMENT

In these tests estrogen, passed from the blood of the mother to the developing egg, has produced results on sexual development in male embryos which are apparently similar (not identical on oviducts) to those earlier observed by Riddle in doves subjected to rapid egg-production (crowded reproduction). These results are also quite comparable with those obtained by others following estrogen injection into the egg of the fowl during early incubation. These findings make it probable that at least a part of the excess of females, and some of the several grades of intersexuality, earlier observed in ring dove offspring by Riddle (1, 2, 23) likewise resulted from excess estrogen in the eggs; the crowded reproduction practiced in those studies should provide extra estrogen along with some other substances (21). In those studies the macroscopic diagnosis of sex was usually made in very early life, in embryos, at hatching, and within 3 weeks thereafter, when the modification of genetic maleness by estrogen is now found to be most pronounced. The conditions observed in figure 12B, particularly if emphasized or if observed at or before hatching, would have been classed in those earlier studies as 'probable female' or as 'female,' but certainly not as 'male.' Both the form and the relative size of the two gonads are sometimes even more 'female' in character than is the histology of the left ovotestis.

Still another probable result of the presence of estrogen in the dove's egg on testis development was earlier indicated by Riddle (24) in the following statement:

> An unexpected disparity of size is found in the testes and ovaries (doves and pigeons) at hatching—the ovary being about twice as large as the testes, although the adult testis is 3 to 5 times as large as the adult (resting) ovary. This is interpreted as a result of the presence of the follicular hormone in the egg, and of its specific action upon the embryonic gonads. This modification of the embryonic growth of organs as the result of the regular, though essentially accidental, inclusion of a maternal hormone within the egg is of interest. The conditions actually found are currently considered normal, though they are quite as abnormal as those effected by the experimentalist.

It may be pointed out that this view is apparently supported by results of Willier

et al. (6) who, in chicks hatched from eggs injected with estrone and estradiol, observed that:

> In the male embryos the *right testis*, although *generally smaller than normal* (our italics) is usually normal in shape and histology; whereas the left testis is very large, has the flattened form of a normal left ovary, and is histologically an ovotestis.

A similar effect on the right testis, which is relatively incapable of developing as an ovary, was not found by these investigators to follow treatment of the early chick embryo with male hormone. They state:

> In the majority of males the right testis is usually normal in *size* (our italics), shape and histology; the left testis in one-third of them is likewise normal and in two-thirds of the cases has more or less the structure of an ovotestis.

Risley (25) noted that in juvenile terrapins estradiol dipropionate brings about a reduction of testis size although it stimulates the rudimentary ovarian cortex of the testis.

The facts observed in this study further lead to the two following considerations. *a*). Intramuscularly injected estrogen permits the dove to dose her eggs more heavily than is usual but probably in quite the same way that she utilizes normally to dispose of an excess of estrogen present in her blood at this time. Riddle (21, 26) demonstrated that a period of greatly increased estrogen production in the dove, as shown by increased oviducal weight, coincides strictly with the final period of 108 hours of rapid growth in the two ova; and Fellner (27) early reported the presence of estrogen in the yolks of eggs of fowl and fish. *b*). Since the observed effect of adding estrogen to an amount already probably normally present in the yolk is to accentuate the amount of ovarian cortex on the left testis, and to prolong its existence, it becomes wholly probable that the smaller amount of estrogen normally present in the yolks of birds and reptiles acts in quite the same manner. We suggest that there is a fair possibility that such estrogen and not a gene is solely responsible for the slight temporary development of ovarian cortex on the embryonic testes of birds and of reptiles (28). The degree of development of Müllerian ducts is evidently likewise at least partly traceable to estrogen in the untreated normal egg.

The question of the extent to which the gonadal hormones affect sex differentiation has been much studied in mammals, and the entire subject has recently been reviewed by Moore (29). The present data provide an indication that estradiol benzoate does not affect the Müllerian duct of the dove embryo in precisely the same way as does the estrogen produced by the dove. The estradiol rather regularly produced in males fluid-filled cysts or remnants, with or without development of the complete duct; but such remnants have probably never been seen, though complete left oviducts in males have frequently been seen, when there was reason to assign their presence to the action of estrogen produced by the bird. Perhaps we can reproduce experimentally the true and full effect of a gonadal hormone on sex differentiation only when we administer a hormone chemically identical with that produced by the species into which it is introduced.

SUMMARY AND CONCLUSIONS

The left testis of the ring dove (*Streptopelia decaocta*, syn. *risoria*) embryo normally shows a slight and temporary development of ovarian cortex which usually persists until hatching (13 of 17 cases) and thereafter soon disappears completely (13 of 14 cases). Normally, Müllerian ducts are inconspicuous and negligible at and after hatching.

Ring dove females were given a single intramuscular injection of 0.5 or 1.0 mg. of estradiol benzoate, or of 2.0 or 3.0 mg. of dehydroandrosterone, 26 to 34 hours before one of their ovarian ova would break from the ovary; during this period some extra estrogen had opportunity to pass from the blood of the mother into this egg-yolk. Most of the 17 genetic males (sex-linked color) obtained from eggs thus treated by the mother herself became intersexes. *a*), Their left testes showed a more extensive and more persistent ovarian cortex; *b*), some, at least, of these left ovotestes also tended to flatten and to become larger (female condition) while the right testes were not markedly modified; *c*), larger and more persistent (atypical) Müllerian ducts developed. Genetic females remained definitely female. Dehydroandrosterone, with very weak estrogenic activity, had at most but little effect (in 2 of 8 cases) on cortical development of the left testis and did not promote Müllerian duct development in males.

These results resemble those of other investigators who injected estrogens into the incubating egg of the fowl. They clearly show that a small or moderate increase of estrogen in the blood of a dove which is maturing eggs is passed from her blood into her ovarian eggs where it may produce intersexuality or sex-reversal in the developing male embryo. Since it was earlier indicated that estrogens are maintained at higher concentrations in the blood of female doves subjected to rapid egg production (21) we now conclude that at least part of the excess of females and of male intersexuality earlier observed by Riddle in offspring of doves subjected to crowded reproduction are ascribable to excess of estrogen passed by the mother into her maturing eggs.

One observed effect of adding some estrogen to an amount probably normally present in the yolk (27) is to increase and prolong the existence of ovarian cortex on the left testis of the dove and chick. It therefore becomes probable that much or all of the evanescent ovarian cortex normally found on the embryonic testes of birds and reptiles arises not through genic action but through action of estrogen.

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BIOLOGICAL PROPERTIES OF PITUITARY INTERSTITIAL-CELL-STIMULATING HORMONE (ICSH)[1]

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I. Effect on the Estrous Cycle of Normal Young Adult Female Rats

IN THE STUDY of the biological properties of purified ICSH, it was desirable to know whether this gonadotropic substance would influence cyclic reproductive phenomena in the female rat. For this reason, therefore, 1 mg. daily or 100 R.U. of pure (homogeneous) ICSH (1) was injected intraperitoneally daily for 10 days into normal adult female rats, 60 days of age. The injections were begun at a uniform time in the cycle, 24 hours after the appearance of cornified vaginal smear. Fresh

TABLE 1. EFFECT OF PURIFIED ICSH[1] ON THE ESTROUS CYCLE AND REPRODUCTIVE SYSTEM OF NORMAL 65-DAY-OLD FEMALE RATS

Group	Estrous Cycle during Treatment	Histology of Vaginal Epithelium	Ovaries		Uterus		Mammary Gland
			Av. wt. mg.	Histology	Av. wt. mg.	Histology	
Experimental (6)	Continuous diestrus	Flattened or columnar; some mucin production in 2 rats	86	Less f. development; c.l. can not be differentiated by age; lipoid content low, evenly distributed	258	Columnar epithelium; open glands or villous projections	Like controls
Controls (6)	Normal 4 to 5 day cycles	Typical of stage of cycle	53	Developing f.; c.l. of different ages. interstitial tissue epithelioid	336	Epithelial lining typical of stage of cycle	Ducts and alveolar buds

[1] Preparation L1149CTP, 100 R.U. per mg.
Total dose 1000 R.U. (10 mg.); intraperitoneally, over a period of 10 days.

corpora lutea were therefore present in the ovary. As will be seen in table 1, the diestrous vaginal picture was maintained almost without exception throughout the 10-day injection period.

The ovaries of the treated animals were slightly heavier (60%), the increase in weight being due to the increase in number and some increase in size of the corpora lutea. Corpora lutea of different ages characteristic of the normal cyclic animal could not be distinguished. The corpora lutea were characterized by a low fat content uniformly distributed as shown by Sudan-Orange stain of frozen sections. The size of the corpora lutea and the size of the cells composing them did not approach the size of the corpora resulting from the injection of lactogenic hormone or estrogenic substances under similar circumstances. The interstitial cells were not enlarged. The number of developing follicles characteristic of the normal ovary was diminished.

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Uteri were definitely smaller than those of the normal cyclic controls. Histologically, the endometrium was characterized by columnar epithelium with wide open glands or villous projections into the lumen. (See figures 1 and 2.) This immediately suggested that the endometrium was under the control of functional lutein tissue and led to the placentoma studies reported in *Part II* of this paper.

Judged by mucin production, the vaginas of the animals did not appear to be completely under the influence of progesterone; in most cases the vagina was lined

TABLE 2. PLACENTOMA FORMATION UNDER THE INFLUENCE OF PURIFIED ICSH[1] IN NORMAL AND HYPOPHYSECTOMIZED 60-DAY-OLD FEMALE RATS. Effects of heating on this property of ICSH

Type of Rat	Material Injected	Total Dose in 10 days intraperitoneally, mg.	Number of Rats	Ovaries, mg.	Uterus, mg.	Placentoma	
						Positive	Negative
Normal	ICSH	10	5	125	583	5	0
	ICSH, heated 1 hour	10	5	134	449	2	3
	ICSH, heated 4 hours	10	5	95	343	0	5
	Control	0	5	67	382	0	5
Hypophysectomized	ICSH	10	5	43	139	0	5
	ICSH, heated 1 hour	10	4	33	133	0	4
	Control	0	7	31	116	0	7

[1] Preparation L1162; 100 R.U. per mg.

with a low epithelium characterized by flattened surface cells. In two cases only was the surface lined by columnar cells containing mucin. The mammary glands showed no development beyond that of the controls.

Summary. Purified ICSH, given in 1 mg. daily doses, prolonged the diestrous interval in normal young adult rats. This effect was associated with the suppression of follicular development and the presence of more lutein tissue than is characteristic of the normal animal. Progesterone secretion by this lutein tissue was indicated by the uterine mucosa but not by the vagina or mammary gland.

II. Placentoma Formation

In a recent paper from this laboratory (2), it was reported that interstitial-cell-stimulating preparations in various stages of purity allowed placentoma formation in normal young mature female rats, with the exception of the purest product (1) tested. This preparation, given at a total dose of 100 units in 10 days was ineffective. All preparations tested were ineffectual in producing the conditions necessary for placentoma formation in similar young adult animals after hypophysectomy.

In the present paper, the pure ICSH has been retested at higher dose levels in both normal and hypophysectomized rats. Further, the capacity to produce placentomata has been tested after destruction of its specific biological properties by heating.

The conditions of the experiments were the same as in those previously reported.

The test animals were young females, 60 days old at the beginning of the experiment, and were chosen so that the experiment was always begun on the day following the finding of a cornified vaginal smear. Fresh corpora lutea were thus insured. Injections were made daily, intraperitoneally, for 10 days. On the day of the 5th injection threads were inserted in the right uterine horn of both injected and control rats in such a way that the endometrium was actually penetrated. Autopsy was performed 24 hours after the last injection and the animals examined for the condition of the ovaries and the presence of placentomata in the uterus. In those cases in which the

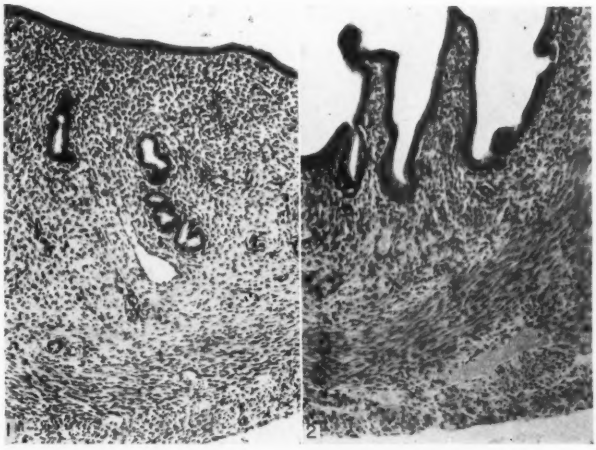

Fig. 1. Uterus of normal 71-day-old rat, in late proestrous stage of cycle.
Fig. 2. Uterus of normal 71-day-old rat injected with purified ICSH, 1 mg. (100 R.U.) daily for 10 days. Note villous projections of the endometrium. The diestrous interval was prolonged throughout the injection period.

animals were hypophysectomized, operation was performed the day after the cornified vaginal smear was observed, and injections were begun on the day of operation.

As will be seen in table 2, a daily dose of 100 R.U. (1 mg.), or a total dose of 1000 R.U. in 10 days, resulted consistently in placentoma formation in normal but never in hypophysectomized rats.

The ICSH after heating was tested at the same dosage as the original material. The protein concentration during heating was 2 mg. per cc. The PH was 7.5. Heating was done in boiling water for 1 or 4 hours. No precipitation occurred after 1 hour but after 4 hours opalescence appeared.

The potency judged by the placentoma tests was reduced almost 60 per cent after 1 hour of heating. The potency of this preparation as judged by the ability to repair the deficient interstitial tissue in the ovaries of hypophysectomized immature rats was also shown to be reduced about 50 per cent after this period of heating. When the preparation was heated for 4 hours, it no longer induced placentoma formation at the specified dose. This length of treatment destroyed almost all of the activity judged by the ability to repair interstitial tissue (i.e. 0.30 mg. of heated ICSH was equivalent to 0.01 mg. of unheated ICSH).

The non-threaded uterine horn of injected animals did not exceed in diameter that of the normal control, even in the group receiving unheated ICSH. The presence of placentomata, however, greatly increased the total uterine weight of responsive

groups. The enlargements themselves were usually discrete swellings at the thread site, measuring 0.5 to 1.0 cm. in diameter as compared with a diameter of only about 0.15 cm. in the unthreaded horn. Histologically, these enlargements showed the typical structure of placentomata. Both uterine horns of animals receiving untreated ICSH showed marked villus formation of the endometrium or wide, open mouths of the glands. When the ICSH was heated one hour, villus formation occurred only in the threaded horn but was found consistently, whereas with the hormone treated 4 hours, villus formation was found in only some of the animals. No such villus formation occurred in either horn of the controls. The threaded and unthreaded horns of

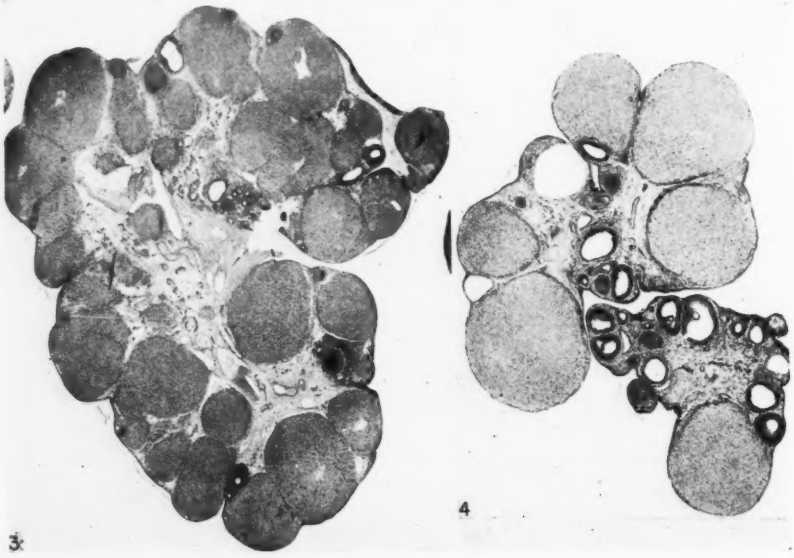

Fig. 3. OVARY OF PREGNANT RAT INJECTED WITH PURIFIED ICSH, 0.5 mg. (50 R.U.) daily, from day 6 through day 21 of gestation. Parturition did not occur, and the animal was killed on day 25. The right and left uterine horns contained 8 and 4 dead fetuses, respectively, average weight 7 gm.

Fig. 4. OVARY OF NORMAL RAT, 20 days pregnant.

the uteri of hypophysectomized rats remained like those of their controls, showing no response in endometrium or myometrium to ICSH injection.

Histological examination of the ovaries of normal animals injected with unheated ICSH showed a decrease in the number of growing follicles. There was some increase in the amount of lutein tissue, chiefly an increase of lutein bodies smaller than normal ovulation corpora. The corpora lutea present at the time of the first injection had not increased in size, nor were the cells of which they were composed larger than normal. (Both of the latter transformations are associated with placentoma formation resulting from lactogenic hormone injection.) After heating of the ICSH, this increase in the amount of lutein tissue was not observed, and no such effects occurred after hypophysectomy. In hypophysectomized rats the only effect of the ICSH was the repair of interstitial tissue.

Although the uterus showed the effect of functional lutein tissue, other evidences were less marked. The vagina, for instance, was never completely under the influence of lutein tissue. When untreated ICSH was injected, the diestrous interval was

prolonged but on injection of ICSH heated for 1 or 4 hours the cycles were almost normal. So also, histology of the vagina showed only mucin production in the group receiving the untreated ICSH, and this in only 3 of 5 rats. The mammary glands in all cases were like those of the controls.

Summary. Pure ICSH induced placentoma formation in normal young rats when given in 1 mg. daily doses. No placentomata resulted from such treatment after hypophysectomy. Heating ICSH solutions for one hour destroyed about one-half of the potency judged by the placentoma test and also by the ability to repair the interstitial tissue of the hypophysectomized animal's ovary. The ovaries of normal rats injected with ICSH showed more lutein tissue, in the form of additional small lutein bodies, but there was no enlargement of corpora lutea, nor any enlargement of lutein cells. It is therefore more probable that ICSH acts by increasing the amount of lutein tissue, rather than by stimulation of the pituitary to pour out lactogenic hormone. The dose of ICSH necessary to obtain this biological effect is high compared with that necessary for the response used to standardize the substance (repair of interstitial tissue).

III. Effect of ICSH on the Duration of Pregnancy and on Parturition in Normal Rats

In view of the fact that the interstitial-cell-stimulating hormone of the pituitary prolonged the diestrous interval of normal rats and allowed placentoma formation, it seemed likely that it would also affect the course and termination of pregnancy in normal rats. For many years it had been known (3) that injection of saline or alkaline extract of beef pituitaries or crude growth hormone preparations would prevent the normal termination of pregnancy in the rat in 21 to 23 days. In such animals, resorbing fetuses were usually expelled by the 25th to 26th day; living young occasionally were born as late as *day 25*. When living young remained in the uterus past term they usually exceeded normal birth weight. It was thought at this time that growth hormone was responsible for prolongation of pregnancy—probably chiefly because growth effects were the only biological effects of these crude extracts known at the time. When, however, growth hormone was purified to the stage attained in 1938 (4), it was found that growth hormone preparations, given in 10 mg. daily doses (capable of producing growth of 62 grams in 20 days in normal adult females) and injected from the 12th to the 21st day of pregnancy did not interfere with the birth mechanism but resulted in litters on *day 23* which were heavier than the normal of the colony, 6.7 vs. 5.6 gm. (confirmation of Watts, 5).

The gonadotropic complex as precipitated by tannic acid also prolonged the gestation period to 25 or 26 days, very few young being born alive; at the time cast they weighed more than normal. The lower ammonium sulfate fractions from pituitary extracts, containing mainly ICSH and no FSH, also resulted in the retention of fetuses past term. At the 25th or 26th day of pregnancy these weighed 7.3 gm. (normal 5.9 gm.). Such preparations were probably not entirely free of lactogenic hormone; it had however been shown that high doses of lactogenic hormone did not inhibit parturition. In one case case a daily dose of 20 mg. (200 I.U.) given from *days 10* to *21* inclusive was followed by normal parturition on *day 24*, litters being of normal or slightly above normal weight, 6.3 gm. The same result followed injection of 20 mg. daily (500–600 I.U.) of lactogenic hormone from *day 15* to 20 of pregnancy except that the litters in this case were born on *day 21* and the young were exactly the weight characteristic for the colony at the time (1936, males 6.1 gm., females 5.9 gm.). The lactogenic hormone preparations used contained some adrenocorticotropin but were free of other physiologically active contaminants (ICSH, FSH, growth and thyro-

tropic hormones). From these unpublished experiments it appeared probable that ICSH might be an important factor, or the factor, in crude or partially purified pituitary fractions, which prevented the normal termination of pregnancy. The present experiment was therefore planned to test the effect of pure ICSH.

A group of 20 normal female rats about 90 days of age were bred, and sufficient time allowed for implantation to occur. On the sixth day after the finding of sperm in the vagina, injections of pure ICSH were begun in 10 rats, 10 being retained as controls. The experimental group received a dose of 0.5 mg. or 50 R.U. daily intraperitoneally, up to and including the 21st day of pregnancy, after which injections were discontinued. All of the controls littered by the 23rd day (5 on *day 22*, 5 on *day 23*). Only one injected rat had given birth to young by the 25th day (2 dead, 1 living). All pregnant animals were therefore autopsied. No living young were found in utero; all had apparently died some hours previously. The young in all cases averaged greater in weight than the average for the normal control litters (6.3 vs. 5.5 gm.).

The ovaries of injected animals, with one exception, were found to contain an abnormal number of corpora lutea (fig. 3, 4). In spite of the presence of so many large lutein bodies, the corpora lutea of pregnancy could, in many cases, be distinguished grossly. The ovaries which proved to be exceptional were those of the single animal able to expel living young. In this case the ovaries contained only the usual number of pregnancy corpora lutea.

Summary. It is evident, therefore, that ICSH must be an important factor in the pituitary which, if unbalanced, leads to the prolongation of gestation in the normal rat, or perhaps more properly speaking, disturbs the parturition mechanism. Occasionally normal young are born as late as the 25th day of pregnancy but the young usually die in utero. The mechanism of action of ICSH in normal pregnant rats may be direct, through excessive luteinization of follicles, though it is still possible it may be indirect, through stimulation of the pituitary to pour out lactogenic hormone.

IV. Effects in the Male Rat

ICSH administered to hypophysectomized male rats causes histological repair of the interstitial tissue of the testis and resumption of the function of this tissue, judged by growth of the male accessory reproductive tissues, particularly of the ventral lobe of the prostate. As will be seen in the following paper on biological methods of standardization, the use of hypophysectomized immature rats in this way affords the basis of one of the most valuable tests for ICSH (6). The poor response of the seminal vesicles to the anterior pituitary ICSH is one of the outstanding enigmas in the study of this substance and is in marked contrast to the action of chorionic gonadotropins. When used after a postoperative interval—i.e., in repair of the testes—ICSH injected in doses as high as 50 R.U. (0.5 mg.) daily for 20 days, has no effect on the tubules except for a slight enlargement unaccompanied by differentiation of the germinal epithelium.

When injected immediately after operation into male rats hypophysectomized after sexual maturity, ICSH will maintain the tubules as well as the interstitial tissue, and the tubules will be retained in functional condition for a period of at least 15 days as is shown by the following experiment. Young mature male rats (77 days of age) were hypophysectomized and injections of ICSH begun on the same day. A high dosage level, 2 mg. or 200 R.U., was injected intraperitoneally for 15 days (6 rats). Two normal control rats were killed at the beginning of the experiment, to show the condition of the testis at the time of hypophysectomy, and 4 hypophysectomized controls were allowed to regress during the 15-day injection period. On the day after

the last injection the rats were autopsied, the epididymis examined for presence and motility of sperm, and the testes and accessories weighed and fixed for histological examination. No evidence of regression could be found upon palpation of the scrotum of the living animals receiving ICSH. The testes remained fully descended, and were large, firm and elastic, as in normal animals. Although the testes of the hypophysectomized controls were not entirely withdrawn from the scrotum, the testes were smaller and softer. The epididymis of injected rats was found to contain innumerable,

TABLE 3. MAINTENANCE OF SPERMATOGENESIS IN ADULT RATS[1] FOR 15 DAYS AFTER HYPOPHYSECTOMY WITH PURE ICSH

Material Injected	Number of Rats	Body Weight		Seminal Vesicles, mg.	Prostate (total) mg.	Testes		Epididymis
		Onset, gm.	Change, gm.			mg.	Histology	
ICSH	5	285	−7	812	589	2944	Tubules almost normal, some decreased in size with desquamating cells; interstitial tissue normal or slightly hypertrophied	Innumerable normal, motile sperm
Hypophysectomized controls. Autopsy	4	244	−44	96	127	972	Tubules not yet regressed to minimum size, still contain adherent spermatocytes, some desquamating; interstitial tissue like connective tissue; nuclei not yet entirely pyknotic	None
Normal Controls. Onset	2	297	—	891	595	3060	Uniform tubules actives with permatogenesis; interstitial tissue epithelioid	Innumerable motile sperm

[1] Rats 76–77 days of age at operation; injections begun the same day and continued for 15 days, autopsy 24 hours after last injection, on day 15 postoperatively. Hypophysectomized controls were autopsied at the end of the experiment, normal controls at the beginning of the injection period. *Preparation L1155*, 100 R.U. per mg., 2 mg. daily intraperitoneally.

normal, motile sperm, not distinguishable in number or appearance from those of normal animals, whereas no sperm remained in hypophysectomized controls. Testis weights were maintained at the level of the normal controls whereas those of the hypophysectomized controls had regressed to less than half this figure (table 3). Histologically, the tubules were seen to have been maintained, although not quite completely. There was some decrease in size and some irregularity of tubules, also desquamation of cells in a few tubules. The interstitial tissue was maintained in the normal, epithelioid condition; there was perhaps even a slight hypertrophy. Seminal vesicles and prostate were likewise maintained, while those of the hypophysectomized controls were greatly reduced.

The maintenance of spermatogenesis seen in such experiments can scarcely be attributed to follicle-stimulating hormone contaminating the ICSH. The contamination with FSH is less than 0.1 per cent. The only explanation of the results which at present suggests itself is that ICSH (resembling human chorionic gonadotropin in

this respect perhaps) maintains the testis by virtue of the testosterone[2] produced by the interstitial cells under its influence. It is accepted that testosterone is capable of maintaining the spermatic tubules of the hypophysectomized rat in functional condition.

Summary. Interstitial-cell-stimulating hormone causes only slight testis-weight increase when used after a postoperative interval (repair), the increase being due to interstitial repair. The tissue is only partially brought back to functional condition judged by the moderate response of the prostate and poor response of the seminal vesicles. When, on the other hand, interstitial-cell-stimulating hormone is given immediately after hypophysectomy to mature males, the size and a functional condition of the tubules and the interstitial tissue are both maintained. The epididymis contains many motile sperm as long as 15 days postoperatively and the accessories are maintained at operative weight.

SUMMARY

Biological properties of interstitial-cell-stimulating hormone as tested in female rats, normal and hypophysectomized:

1. Injected into the adult normal rat, ICSH prolongs the diestrous interval.
2. It increases the amount of lutein tissue in normal rats, not in hypophysectomized rats. (The corpora lutea produced in normal rats appear to be functional, as judged at least by 3, 4 and 5, below.)
3. Uteri of normal rats injected with ICSH are characterized by wide mouthed glands and villous protusions of the endometrium.
4. Placentoma form about threads inserted in the uteri of normal rats injected with interstitial-cell-stimulating hormone, but not in hypophysectomized females so treated from the time of operation.
5. Some mucification of the vagina occurs in normal rats injected with interstitial-cell-stimulating hormone but not in hypophysectomized rats.
6. Pregnancy in normal rats is prolonged and the birth of living young prevented by interstitial-cell-stimulating hormone.

Biological properties of interstitial-cell-stimulating hormone as tested in hypophysectomized male rats:

1. ICSH in hypophysectomized males repairs the interstitial tissue; some accessory development also occurs, especially in the ventral prostate but no tubular stimulation results.
2. ICSH, if given immediately after hypophysectomy, will maintain sperm formation in the adult male rat. The interstitial tissue remains active judged both by histological appearance and by maintenance of the accessory system (prostate and seminal vesicles).

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[2] Adrenals had not regressed in weight as far as those of the hypophysectomized controls, averaging 25 mg. (normal, 47 mg.; hypophysectomized controls, 15 mg.). Histologically, the adrenals were seen to vary greatly, from almost complete maintenance to regression nearly as great as that of the controls. This may not have been evidence of contamination of the ICSH with ACTH, but evidence rather of maintenance by sex steroids produced by the testes under the influence of ICSH. Similar, though more complete, maintenance of the adrenals by testosterone has recently been observed by as in this type of experimental animal.

COMPARISON OF METHODS FOR STANDARDIZATION OF PITUITARY INTERSTITIAL-CELL-STIMULATING HORMONE (ICSH)[1]

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I. Repair of Ovarian Interstitial Tissue in the Hypophysectomized Rat

THE METHOD that has been used constantly for the standardization of ICSH preparations in this laboratory for the last 8 years, and which has been adequate to allow for the preparation of ICSH in pure form (1), is the repair of the ovarian interstitial tissue of the hypophysectomized rat. The rats are used at a standard age (26–28 days) and postoperative period (6–8 days). The routine injection procedure is once daily, intraperitoneally for 3 days, followed by autopsy 72 hours after the first injection. At this time the animals are killed, the ovaries are observed under the binocular at a magnification of 10×, dissected weighed and sectioned for histological examination. The only effect of such injection on the ovary observable macroscopically is an increased size and vascularity, the ovary appearing pink rather than white as in the hypophysectomized control. The weight increases are not large, the ovaries not being distinguishable by weight from those of the control at minimum dosage levels, while at high dosage levels, pure ICSH will produce an increase from the control weight of 9 mg. to a weight of 30 mg.

On microscopic examination of the ovaries the increased weight is seen to bedue almost entirely to stimulation of the interstitial cells (although an increase in the blood vascular beds also accounts for some of the weight increase). The changes in the interstitial cells under the influence of pure ICSH are not illustrated here but have been given in earlier publications (see fig. 2 of ref. 2). The minimum effect on this tissue shows itself in a slight increase in size of the nucleus which becomes more rounded; the chromatin, which in the control has been in discrete depots, becomes evenly scattered throughout the nucleus; the nucleus therefore takes a lighter hematoxylin stain. The cytoplasmic body, which is scarcely discernible in the deficient interstitial tissue of the hypophysectomized control increases in amount and becomes more eosinophilic; the outline of the cell becomes more distinct. By such transformations cells which had degenerated to a condition simulating connective tissue cells, resume an epithelioid appearance. Interstitial cells are, however, even at high dosage levels, rarely as large or as pronouncedly eosinophilic as are these cells after moderate doses of chorionic gonadotropin.

Beginning repair, as judged by these criteria, is shown under the conditions of injection just specified, when a total of 0.005 mg. of pure sheep pituitary ICSH is injected intraperitoneally. If the subcutaneous route of injection is used, the test is one-fifth as sensitive, the response being given only at 0.025 mg. This 1 to 5 ratio is the same as given for earlier preparations (3).

The Leydig tissue of the testis appears to be about as sensitive to ICSH as the interstitial tissue of the ovary and therefore can also be used in testing for ICSH.

[1] Aided by grants from the Research Board of the University of California, from the Rockefeller Foundation, and from Parke, Davis and Company. Assistance was rendered by the Work Projects Administration, Official Project No. 265-1-08, Unit A-5.

II. Augmentation of FSH by ICSH

A much less commonly used test, but one which is even more sensitive than the one just described, is afforded by the augmentation of the effects of FSH when it is injected in combination with ICSH. If, for example, 2 to 3 R.U. of FSH (hypophysectomized rat units) is injected into the hypophysectomized female rat of the type described in Section I, a small amount of follicular growth results, that is, small and medium follicles develop in the ovary within 72 hours. This development is, however, not accompanied by an estrous condition of the uterus, a dose twice this size being

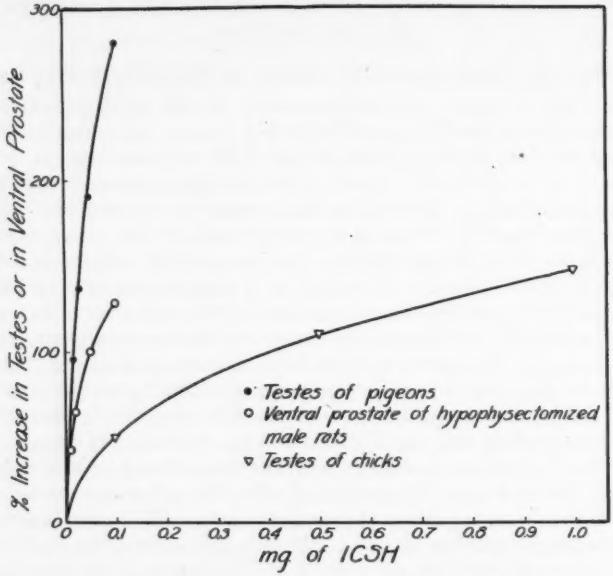

Fig. 1. COMPARISON OF SENSITIVITY of quantitative methods for biological assay of ICSH.

necessary to cause estrus. If, however, this dose of FSH is combined with 0.001 mg. only of pure ICSH and the mixture is injected subcutaneously, not only greater follicular development results, but an estrous condition of the uterus follows. Therefore by injection subcutaneously of combinations of ICSH with FSH, 0.2 R.U. can be recognized or $\frac{1}{5}$ the amount of ICSH detectable by repair of the interstitial tissue when injected intraperitoneally. The precaution should be observed in this test to have relatively pure FSH, as it must be remembered that any contaminating ICSH in the FSH preparation would cause the appearance of an estrous uterus at a lower unitage.

III. Increase in the Weight of the Accessory Sex Organs of the Hypophysectomized Male Rat

The prostate and seminal vesicles of the hypophysectomized male rat have frequently been used in measuring the potency of the second hypophyseal fraction. Recently, Greep et al. (4) have pointed to the relatively greater reactivity, and therefore greater importance of the ventral lobe of the prostate for measurement of pituitary ICSH. They used the hypophysectomized immature male rat (21 days old at operation, 2 days postoperative at beginning of injections). The material to be as-

sayed was injected subcutaneously daily for 4 days with autopsy on the 5th day, 24 hours after the last injection.

We can confirm that the weight increase of the ventral prostate is a valuable measure of ICSH potency, whereas the increase in seminal vesicle weight is relatively useless in the standardization of pituitary ICSH (5). It will be noted in table 1 that between 0.05 and 0.10 mg. of ICSH injected into such rats increases the weight of the ventral prostate approximately 100 per cent, whereas the seminal vesicle weight is practically unaffected at such doses or even multiples of them. The test becomes even more sensitive when injections are made intraperitoneally instead of subcutaneously as specified by Greep et al. It will also be noted in table 1 that the testis

TABLE 1. EFFECT OF SEVERAL PURIFIED ICSH PREPARATIONS IN HYPOPHYSECTOMIZED MALE RATS.[1] SUMMARY

Preparations	Total Dose per Rat, mg.	Number of Rats	Average Weight				
			Ventral Prostate		Seminal vesicles, mg.	Testes mg.	Body weight, gm.
			mg.	% increase			
L1063 TP	0.10	5	19	137	8	189	56
	0.05	5	17	111	7	159	54
	0.025	15	14	75	7	156	56
	0.01	10	12	50	7	138	53
L1066 TP	0.10	4	19	137	7	168	50
	0.02	4	14	75	8	145	52
L1087 BS	0.50	5	24	200	10	161	52
	0.10	7	18	125	9	162	53
	0.02	5	12	50	6	146	53
L1120 B	0.5	4	23	187	10	184	50
	0.1	9	19	137	8	174	50
	0.02	8	13	60	7	137	50
	0.10[2]	10	21	162	14	159	52
L1137 CP	0.10	6	18	125	9	201	57
	0.02	11	14	75	10	156	56
Control	0.0	36	8		8	121	53

[1] 23 days old, 2 days postoperative at start of injection, injected subcutaneously daily for 4 days, with autopsies on 5th day.

[2] Intraperitoneally injected, instead of subcutaneously.

increases somewhat in weight but never exceeds 200 mg. (control 121 mg.) even at the 0.5 mg. dose level.[2]

IV. Maintenance of Testes and Accessories of Hypophysectomized Mature Male Rats

Maintenance of testes and accessory reproductive organs after hypophysectomy of mature males presents a different problem. The testis of such animals, already functional at hypophysectomy, is maintained in functional condition, without decrease in size of tubule or decrease in sperm production, by daily doses of 2 mg. of ICSH. Under these conditions, seminal vesicles as well as prostate are completely maintained. The interstitial tissue of the testis is morphologically normal and ap-

[2] The inability of sheep ICSH preparation to cause greater increase of testes weight is in keeping with the findings of Greep et al using pig ICSH and may be considered to be further evidence of freedom of sheep ICSH from FSH. FSH injected into this test animal causes testis weight increases proportional to the dose.

TABLE 2. SUMMARY OF THE EFFECT OF ICSH IN IMMATURE PIGEONS

Type of Pigeon	Length of Injection	Total Dose	Number of Pigeons	Testes	
				Weight	Increase
	days	mg.		mg.	%
Normal	4[1]	0	25	25	
		0.015	3	40	60
		0.030	3	50	100
		0.05	3	61	144
		0.10	2	80	220
		0.25	2	124	396
		0.50	2	169	576
		1.0	3	114	356
		2.0	3	125	400
	10	0	2	42	
		10.0	2	240	472
Hypophysectomized[2]	10	0	9	24	
		10.0	4	250	942

[1] Pigeons of a constant strain, Silver King, 33 ± 3 days old at start of injection, were injected intramuscularly once daily for 4 days; autopsied 96 hours after the first injection.

[2] Pituitaries were removed at 35 days of age, regression was allowed for 14 days; normal pigeons receiving 10 daily injections were 35 days old at start of injection.

pears to be secreting sex hormone. Evidently we are here concerned with problems similar to those raised by the maintenance of spermatogenesis in the absence of the pituitary by testosterone. Maintenance of testis or accessories by ICSH has not yet been standardized adequately to propose a unit, but the method is promising (see discussion of biology of ICSH, ref. 6, table 3).

TABLE 3. A SUMMARY OF RESULTS OF ICSH ASSAY IN CHICKS[1]

Preparation	Total Dose per Chick	Number of Chicks	Testes	
			Weight	Increase
	mg.		mg.	%
L1137CP	5.0	6	23.4	342
	1.5	5	16.4	209
	1.0	10	12.6	138
	0.5	4	10.5	98
	0.1	6	7.5	42
L1149C	5.0	7	22.8	330
	1.0	7	12.7	140
	0.5	5	12.0	126
	0.1	6	8.6	62
Control	0	6	5.3	

[1] White Leghorn chicks, one day old, were injected subcutaneously with 0.2 cc. daily for 5 days; autopsied 24 hours after the last injection.

V. *Weight Increase of Testes of Immature Pigeons*

It has been known for many years that the testis of the immature pigeon is very sensitive to pituitary gonadotropins (7–10). Recently Lahr et al. (11) studied histologically the development of the pigeon testis after treatment with various gonadotropins but no quantitative data are reported as to the relationship between the dose

of ICSH and the weight response. Fischer (unpublished thesis, University of California, 1939) found increase in weight of the testis, caused only by hypertrophy of interstitial tissue, in hypophysectomized immatured pigeons of this age with 10 R.U. of ICSH of the greatest purity then obtainable (the minimal effective dose for repairing interstitial tissue was 0.025 mg.). In a later report, Schooley et al. (12) cast doubt on the ability of pure luteinizing hormone (ICSH) to stimulate the testis of normal or hypophysectomized pigeons.

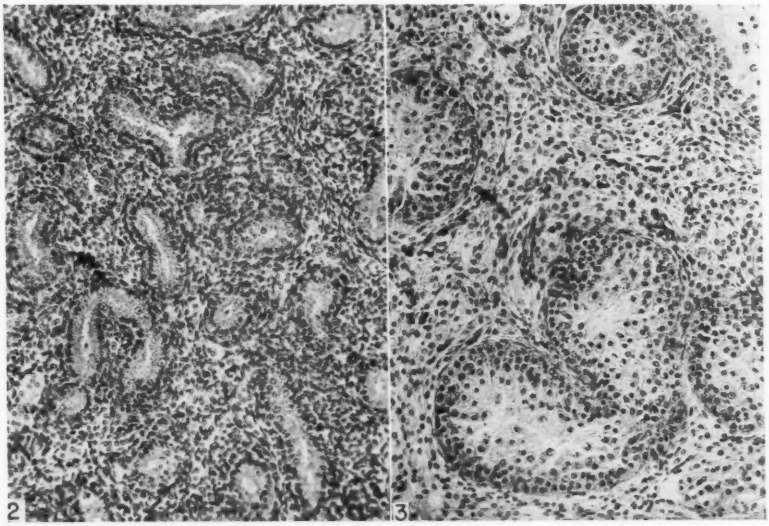

Fig. 2. TESTIS OF NORMAL 45-DAY-OLD PIGEON. Tubules are small, have almost no lumen, and are lined with columnar or cuboidal epithelium. Individual interstitial cells are not clearly circumscribed, and occur in groups of 5 or 6 cells. Weight of 2 testes, 39 mg. H. & E. ×140.

Fig. 3. TESTIS OF NORMAL 45-DAY-OLD PIGEON after injection over a 10-day period of 1 mg. daily of ICSH. Interstitial cells have become larger and more eosinophilic, occurring in compact cords between the tubules. The tubules are larger and show differentiation of lining cells; 3 or 4 layers of adherent cells are present. A peculiar filamentous cytoplasm streams inward from these cells almost obliterating the lumen. Weight of 2 testes, 320 mg. H. & E. ×140.

The data in table 2 show the sensitivity of the pigeon's testis to the pure sheep ICSH. A total dose of approximately 0.03 mg. in 4 days is sufficient to increase the testis weight of normal birds 100 per cent; when about 10 times this dose is given a maximum increase of testis weight was obtained. The data in table 2 show also that the testis of hypophysectomized pigeons is highly sensitive to the interstitial-cell-stimulating hormone.

Histologically it could be seen that the weight increase of both normal and hypophysectomized pigeon testes was due largely to hypertrophy and hyperplasia of the interstitial cells of the testis (4 to 10 day injection period). Some increases of the tubular diameter occur and even some differentiation of tubular epithelium at high doses after 10 days injection. Figures 2 and 3 show the results for 10 daily injections of ICSH into normal pigeons, figures 4, 5 and 6 show the results of the same treatment of hypophysectomized pigeons; for further detail see the legends.

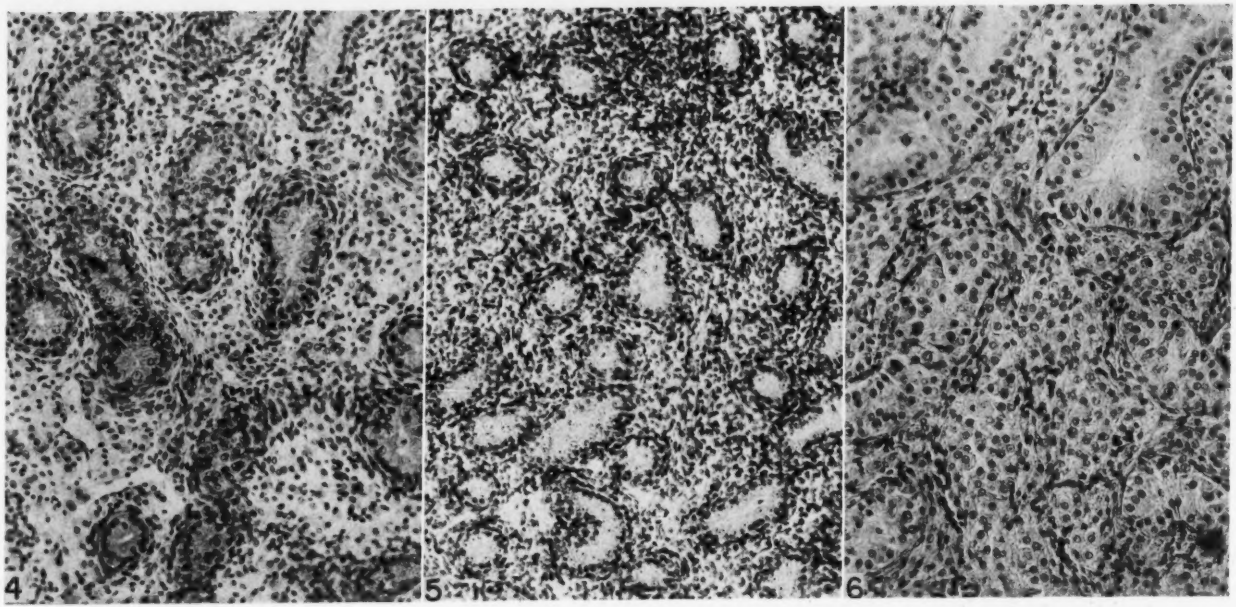

Fig. 4. TESTIS OF NORMAL 59-DAY-OLD PIGEON. Tubules are still small and undifferentiated. Interstitial cells occur in small, isolated groups. Weight of 2 testes, 36 mg. H. & E. ×180.

Fig. 5. TESTIS OF UNINJECTED HYPOPHYSECTOMIZED PIGEON, 49 days old, 15 days postoperatively. Note shrunken tubles and scattered groups of small interstitial cells. Weight of 2 testes, 24 mg. H. & E. ×180.

Fig. 6. TESTIS OF HYPOPHYSECTOMIZED PIGEON, 59 days old, 14 days postoperative at start of injection with ICSH, 1 mg. daily for 10 days. Note compact cords of hypertrophic interstitial cells. Tubules are enlarged, but not differentiated and the lining epithelium shows filamentous cytoplasm. Weight of 2 testes, 218 mg. H. & E. ×180.

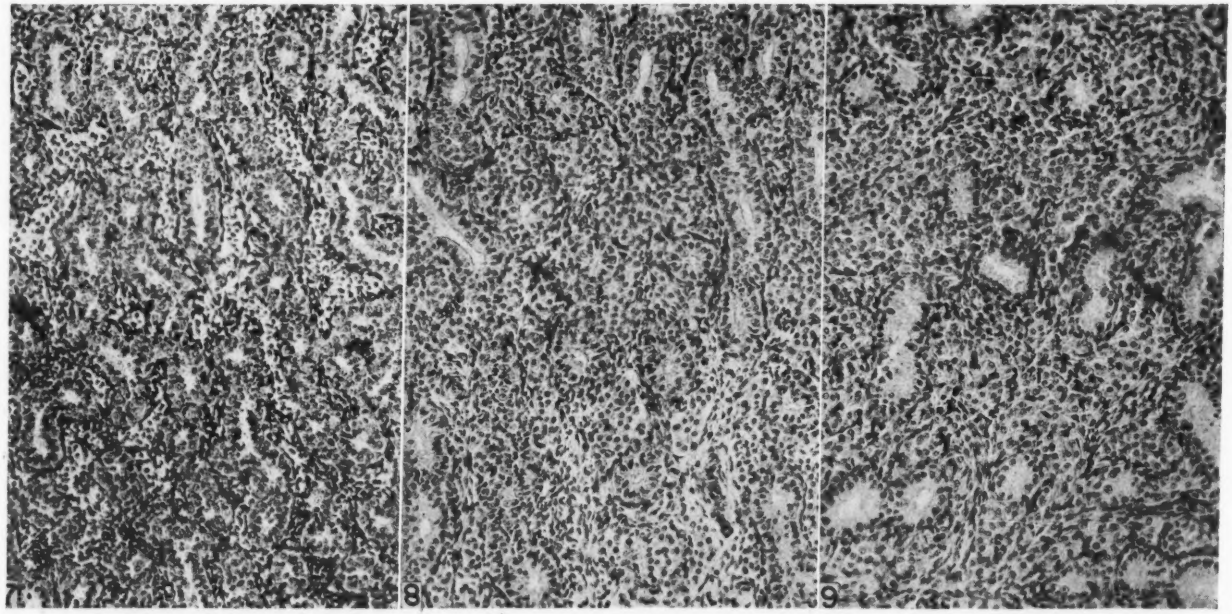

Fig. 7. Testis of 6-day-old white leghorn chick. Note small, tightly packed tubules, lined by cuboidal epithelium and showing almost no lumen. Interstitial cells are small and indistinctly outlined, occurring in rows of 2-3 cells between the tubules. H. & E. ×180.

Fig. 8. Testis of 6-day-old white leghorn chick injected from day 1 for 5 days with a total dose, of 0.1 mg. of ICSH. This figure shows the minimum detectable histological effect. Number of rows of cells in cords of interstitial cells between tubules not increased but individual cells larger and more eosinophilic. H. & E. ×180.

Fig. 9. Testis of 6-day-old white leghorn chick injected from day 1 for 5 days with a total dose of 5.0 mg. of ICSH. Interstitial cells large and rounded with central nucleus surrounded by a mass of eosinophilic cytoplasm; occur in cords of 5–10 cells between tubules. Tubules are undifferentiated, lined by columnar cells with granular, filamentous cytoplasm which practically obliterates the lumen. H. & E. ×180.

VI. Weight Increase of Testes of One-Day-Old Chicks

The testis of the one-day-old chick is also known to be very responsive to gonadotropins, increasing in weight markedly just as do the testes of the immature pigeon. Breneman (13) found an increase of the interstitial tissue in testes of immature chicks injected with luteinizing hormone even in cases where the testis weight was only slightly greater than that of uninjected chicks. Smelser (14) confirmed these observations and noted no synergism between FSH and ICSH in this type of animal. More recently Bates et al (15) observed a relationship between dose and response in testes of chicks injected with a gonadotropic preparation containing FSH and ICSH.

As shown in table 3, the one-day-old White Leghorn chicks injected for 5 days, daily, subcutaneously, responded with a 100 per cent increase in testis weight at a total dose of 0.5 mg. of pure sheep ICSH. Further weight increases occurred as the dose was increased, at least up to 10 times this level. The testes showed on microscopic examination that the increase in weight was due almost entirely to interstitial tissue hypertrophy and hyperplasia (fig. 7, 8, 9). The tubules were just perceptibly increased in size (but showed no differentiation) at high dosage levels. The effect of a total dose of 5 mg. is shown in figure 9. This result is to be compared with the control shown in figure 7. Figure 8 shows that the interstitial cell enlargement is still discernible histologically at a total dose of 0.1 mg. i.e. at 0.2 the dose necessary to produce 100 per cent weight increase. Marked hypertrophy and hyperplasia of this tissue is shown at the higher level (fig. 9).

SUMMARY

Comparison of the assay methods. The methods for assay of ICSH which have been put on a quantitative basis, namely testes weight increment in chicks and pigeons and the increase in ventral prostate weight of hypophysectomized male rats, are compared in figure 1. It may be seen that the most sensitive of these tests is the response of the pigeon's testis, for which the minimal effective dose is 0.030 mg. The response of the ventral prostate is the next most sensitive test (0.05 to 0.10 mg.) and the chick testis is the least sensitive (0.5 mg.). Either qualitative test, the augmentation test or determination of the minimum effective dose for repair of interstitial tissue in hypophysectomized immature female rats, is more sensitive than the quantitative methods, the former being given at 0.001 mg. the latter at 0.005 mg.

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MAMMOGEN AND UNILATERAL MAMMARY GLAND GROWTH IN THE RABBIT[1,2]

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AS A RESULT of the observation that the ovarian hormones were unable to stimulate the growth of the mammary gland in hypophysectomized animals (1, 2) and, further, that anterior pituitary tissue from pregnant or estrogen-treated animals would cause mammary gland hyperplasia in hypophysectomized animals (2), the theory was advanced that the ovarian hormones acted upon the mammary glands indirectly by way of the pituitary. It was suggested that two mammogenic hormones were secreted by the pituitary. Mammogen *I*, the duct growth factor, was stimulated to increased secretion by the estrogens whereas, mammogen *II*, the lobule-alveolar growth factor, was stimulated by progesterone. The extensive literature concerning this problem has been reviewed recently (3).

Several investigators have reported that percutaneous application of estrogen is capable of causing growth of the duct system of the mammary glands lying below the treated area whereas untreated glands remained unaffected or at least less extensively developed (4–9). To some this work could be explained only by assuming the direct action of estrogen upon the mammary gland (7, 8, 9) whereas to others it indicated merely that estrogen was playing an additional direct rôle in the metabolism of the stromal tissue of the mammary glands, synergizing the action of the pituitary factors (10); this especially, since percutaneous application is ineffective in the hypophysectomized animal (11, 12).

As early as 1931, it was shown by work in this laboratory that the stimulation of lobule-alveolar growth by the administration of progestin was facilitated by the simultaneous injection of estrogen (13). More recently, however, Selye (14) has shown that large doses of progesterone alone were sufficient to stimulate lobule-alveolar growth. Mixner and Turner (10), using a new assay method, have reported an extensive study of the relation of estrogen to progesterone and related sterols as well as to anterior pituitary extracts which have the ability to stimulate lobule-alveolar growth. They reported that five or six times as much progesterone or related compounds were required in the absence of estrogen. Similarly, several investigators have indicated that the combination of pituitary extracts and estrogen in hypophysectomized animals was more effective in stimulating mammary duct growth than pituitary extracts alone (15, 16).

As a result of these observations, the suggestion was made (10) that estrogen played a dual rôle in stimulating the growth of the mammary gland. In addition to its action upon the pituitary in stimulating increased secretion of mammogen *I*, estrogen might also play an important function in producing a hyperemic condition in the stromal tissue surrounding the growing duct system as well as increasing the permea-

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bility of the capillaries, as occurs in the uterus (17, 18, 19). Increasing the availability of the effective hormone and nutrients to the growing epithelial cells would provide for optimal growth. In a similar manner, the synergism of progesterone and estrogen could be explained. Without the local action of estrogen the required amount of progesterone to cause lobule-alveolar development was shown to be greatly increased. While these interrelations have now been well established (10), it is more difficult to separate the two suggested rôles of estrogen in stimulating duct growth.

If a substitute could be found for the effect of the local application of estrogen to the skin, namely local hyperemia and increased capillary permeability, non-estrogenic substances would also be effective, when applied locally over the mammary glands, in stimulating growth of the duct system. In this case it is assumed that sufficient mammogen *I* is circulating in the blood stream but that lack of duct growth is due to the absence of the local action of estrogen upon the gland stroma.

Recently, Hooker and Williams (20) reported that the application of spirits of turpentine to the nipples and surrounding skin caused a retardation in the rate of involution of the mammary alveolar system in mice from which the young had been removed. Mixner and Turner (21) confirmed their observations but were unable to show a stimulation of the growth of alveoli in either castrate or normal female mice. As the turpentine treatment of the skin over the mammary glands caused great subcutaneous hyperemia, it seemed desirable to determine whether turpentine and other skin irritants might influence the mammary stromal tissue as does the local application of estrogen.

PROCEDURE

The skin on the ventral body wall of male New Zealand White rabbits was shaved from the jaws to the inguinal region, exposing the teats. In most of the animals the hair was removed unilaterally for treatment of one row of mammary glands. The hair remaining on the unshaved side then served as a barrier to the fluid applied to the shaved side. In several rabbits to which estrogen was applied unilaterally the carrier oil or alcohol was applied to the control side. Estrone was dissolved in olive oil and applied once daily to the skin around each teat on one side while the irritant oils were applied as indicated by the condition of the skin. As the hair regrew from the shaved skin, it was removed by reshaving. With some of the irritant oils the condition of the skin made it impossible to reshave. Three hundredths to .05 cc. of oil or alcohol carrier was applied each day to each teat or a sufficient amount to spread over the gland area with slight rubbing.

On termination of the experimental period, the skin was dissected off and the mammary glands on each side were removed in a single strip with accompanying muscle and connective tissue. The glands were fixed on cork in Bouin's fluid, stained in Mayer's hematoxylin after removing muscle and surplus connective tissue. The glands were then examined and measured under the dissecting microscope. Negative or unresponsive glands were considered to be those with short rudimentary ducts hardly extending beyond the base of the teat.

RESULTS

Two rabbits given 6 I.U. of estrone per treated teat per day for 30 days had well-developed mammary duct systems (table 1). Both the treated and untreated glands were developed but the treated glands averaged 20 and 17 per cent longer than the untreated.

Six rabbits were given 3 I.U. of estrone per teat per day as recommended by Lyons and Sako. In three cases no control glands were developed while treated glands

TABLE 1. PERCUTANEOUS TREATMENT OF MALE RABBITS FOR MAMMARY GLAND GROWTH

Weight, gm.	Days Treated	Subcutaneous Chemical, Daily Dose	Percutaneous Chemical, Daily Dose; 1 Teat	Carrier	Size of Glands, Centimeters Treated	Untreated
2650	30	none	Estrone, 6 I.U.	oil	(4) 6.6×2.7	(4) 5.4×2.5
2430	30	none	Estrone, 6 I.U.	0.03 cc. per teat	(4) 4.1×2.3	(4) 3.5×1.6
2600	30	none	Estrone, 3 I.U.		(4) 2.8×1.4	(3) 1.9
2190	30	none	Estrone, 3 I.U.		(3) 2.0	(2) negative (1) 1.2
3220	30	none	Estrone, 3 I.U.		(4) 1.9×1.3	(3) negative (1) 1.1×0.9
2900	30	none	Estrone, 3 I.U.		(3) 1.6×0.8 (1) negative	(4) negative
3055	30	none	Estrone, 3 I.U.		(4) 2.1×1.3	(4) negative
2925	30	none	Estrone, 3 I.U.		(2) 2.5×1.5 (2) negative	(4) negative
2800	60	none	Estrone, 3 I.U.		(4) 5.5×2.5	(4) 2.7×1.6
3060	60	none	Estrone, 3 I.U.		(4) 3.7×1.6	(3) negative (1) 2.0×0.9
2330	30	none	Diethylstilbestrol, 2.0 μg.	Alcohol	(4) 4.7×2.7	(4) 3.9×2.4
2181	30	none	Diethylstilbestrol, 0.2 μg.	0.03 cc. per teat	(4) 4.9×2.3	(5) 3.3×1.9
2935	30	none	Diethylstilbestrol, 0.1 μg.	0.03cc. per teat	(4) 2.3×1.6	(3) negative (1) 1.1×0.6
2915	30	none	Diethylstilbestrol, 0.1 μg.	0.03cc. per teat	(2) 1.7×1.4 (2) negative	(4) negative
2637	30	none	Diethylstilbestrol, 0.02 μg.	0.03 cc. per teat	(3) 1.2×0.8 (1) negative	(1) negative (3) 1.0×0.9
2235	30	Estrone, 20.0 I.U.	Paint turpentine		(4) 4.6×2.3	(4) 4.1×2.0
2650	30	Estrone, 10.0 I.U.	0.05 cc. per teat		(4) 4.7×1.8	(4) 4.1×2.4
3167	30	Estrone, 5.0 I.U.	0.05 cc. per teat		(4) 2.7×1.8	(3) 2.2×1.2
2593	30	Estrone 2.5 I.U.	0.05 cc. per teat		(3) negative	(3) negative
2413	30	Estrone, 0.5 I.U.	0.05 cc. per teat		(4) negative	(4) negative
2415	30	Diethylstilbestrol, 0.4 μg.	Paint turpentine		(4) 3.7×2.0	(4) 3.6×1.9
2345	30	Diethylstilbestrol, 0.2 μg.	0.05 cc. per teat		(4) 4.1×1.9	(4) 3.8×2.2
2080	30	Diethylstilbestrol, 0.1 μg.	0.05 cc. per teat		(4) 2.3×1.5	(3) 3.1×1.7
3000	30	Diethylstilbestrol, 0.05 μg.	0.05 cc. per teat		(4) 2.2×1.4	(3) 2.0×1.3
2650	30	Diethylstilbestrol, 0.05 μg.	0.05 cc. per teat		(4) 1.9×1.3	(4) 2.1×1.6
3000	30	Diethylstilbestrol, 0.025 μg.	0.05 cc. per teat		(2) 1.1 (2) negative	(2) 0.9 (2) negative
2940	30	Diethylstilbestrol, 0.025 μg.	0.05 cc. per teat		(4) negative	(4) negative
2238	30	Diethylstilbestrol, 0.025 μg.	0.05 cc. per teat		(4) negative	(4) negative
3200	30	none	Paint turpentine		(3) 1.4×1.0 (1) negative	(4) negative
3400	30	none	Paint turpentine		(4) negative	(4) negative
2490	30	none	Paint turpentine		(4) negative	(4) negative
2530	30	none	Paint turpentine		(4) negative	(4) negative
3130	30	none	Oil of myrrh		(4) negative	(4) negative
2735	30	none	Oil of myrrh		(4) negative	(4) negative
2400	30	none	Medical turpentine		(4) negative	(4) negative
2650	30	none	Medical turpentine		(4) negative	(4) negative
2398	24	none	Oil of Cantherides		(4) negative	(4) negative
2140	30	none	Eucalyptus oil		(4) negative	(4) negative
1910	30	none	Eucalyptus oil		(4) negative	(4) negative

averaged from 2.5×1.5 cm. to 1.6×.8 cm. The other three rabbits had from one to three slightly developed glands on the untreated side while on the treated side all glands were from slightly to fairly well developed (table 1).

Two rabbits were given 3 I.U. per teat for 60 days. In one case the treated glands averaged 3.7×1.6 cm. Only one control gland was developed. In the second case the treated glands averaged twice as long as the controls.

Localized mammary development was also attempted with diethylstilbestrol percutaneously applied. In one rabbit given 2.0 μg. per teat per day in alcohol the treated glands averaged 20 per cent longer than the untreated, although both sets were well developed. A rabbit given 0.2 μg. per teat had treated glands averaging 49 per cent longer than those of the controls. Of two rabbits given 0.1 μg. per teat, one showed no development of the control glands while two of the treated glands were somewhat developed averaging 1.7×1.4 cm. The other rabbit had a fair development of the treated glands (2.3×1.6 cm.). Three of four control glands were negative in their response. Another rabbit given 0.02 μg. per teat had three glands on each side slightly developed.

Three male rabbits were given daily injections of 5, 10, and 20 I.U. of estrone for 30 days. At the same time the shaved skin on one side of the thorax and abdomen was maintained in a highly inflamed condition by frequent application of crude turpentine. The turpentine-treated glands averaged 23, 15 and 12 per cent longer respectively than the controls. In one case, however, the area of the control glands was equal to that of the treated ones.

Five rabbits given .05 to 0.4 μg. of diethylstilbestrol daily by injection and treated percutaneously with turpentine unilaterally did not show any tendency for the turpentine-treated mammary glands to be larger than the untreated glands. Glands from those rabbits given 0.025 μg. per teat per day of diethylstilbestrol showed from slight to no development.

When turpentine was applied alone, unilaterally, the results were negative in most cases. However, in one case, three of four treated glands averaged 1.4×1 cm. while untreated glands were negative in their response. Negative results were secured with percutaneous application of oil of myrrh, medical turpentine, oil of cantherides and eucalyptus oil.

DISCUSSION

We were able to confirm the results of Lyons and Sako with estrone unilaterally applied to the shaved skin of the male rabbit. Six I.U. per teat per day was too much, causing extensive development of mammary duct systems in both treated and untreated glands. Treated glands were somewhat larger, however. Three I.U. per teat per day for 30 days gave the desired results in half of the cases, but treated glands were rather small and not well developed. The same dosage applied for 60 days caused the development of larger mammary duct systems on the treated side, but some of the untreated glands developed somewhat.

Diethylstilbestrol also caused unilateral mammary duct development. Dosages of 2 μg. and 0.2 μg. per teat per day were too high, causing only a differential rate of growth of treated and untreated glands. Better results were secured in two rabbits given 0.1 μg. per teat per day. These results are probably similar to those reported by Lyons and Sako for estrone, although the latter authors reported only two successful cases, classing the rest as preliminary results.

It was thought that, on injection of an estrogen, the irritative qualities of turpentine might cause an increased rate of proliferation of treated glands over that of controls. There may have been some tendency for this to occur in rabbits injected with estrone since two of three with mammary gland development had larger glands on the turpentine-treated side. In a large group of rabbits given injections of diethylstilbestrol there did not appear to be any tendency for the turpentine-treated glands to be larger.

When turpentine applied unilaterally was the only treatment, one rabbit had three mammary glands with duct systems which had apparently developed while all the untreated glands were rudimentary. This looked as though turpentine alone might cause mammary gland development presumably by its hyperemic and irritative qualities, but further trials were negative. No response was secured with several other irritants.

These attempts to find a non-estrogen which would cause unilateral mammary gland growth by reason of its ability to increase the blood supply and/or permeability of the capillaries of the stromal tissue of the mammary glands have been inconclusive or negative. With some other substance or technic it may be possible to demonstrate that the localized action of estrogen on the mammary gland is due to its effect on the nutrition of these tissues.

SUMMARY

Unilateral percutaneous treatment of male rabbits with estrone or diethylstilbestrol caused either greater development of the mammary glands on the treated side, or in some cases, development of treated glands only. Injection of estrone and unilateral percutaneous application of turpentine caused, in two of three rabbits, the development of larger mammary glands on the turpentine-treated side. Turpentine-treated mammary glands from diethylstilbestrol-injected rabbits did not show an increased size over untreated glands. One rabbit, given turpentine alone, unilaterally had several mammary glands showing some duct development on the treated side. This could not be repeated in other rabbits. Other irritants applied unilaterally to the shaved skin gave negative results.

It appears probable that estrogens possess characteristics and properties not present in skin irritants which superficially appear to cause local subcutaneous hyperemia.

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RESPONSES OF THE TESTIS TO ANDROGENIC TREATMENTS[1]

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THE EFFECTS OF MALE HORMONE on the testicle were first studied by Moore and Price (1, 2). Rats were injected with testis concentrates at physiological levels, that is, sufficient androgenic material was administered to maintain the accessory reproductive organs of castrate rats in a normal condition.

Previous investigators (3–6) had noted that injections of estrogenic substances into normal males produced serious testicular damage, including reduction in testis size, marked reduction in weight, degeneration of germinal epithelium, and prevention of spermatozoon formation. The general interpretation of such effects had been based largely on the idea of an 'antagonistic reaction' between the sexes or an 'antimasculine influence.'

Moore and Price noted that, in young growing males in particular, introduction of androgenic substances led to testicular damage of a similar character to that described after estrogenic treatment. Since this could not represent an antagonistic influence, in the sense that secretions from opposite sexes were mutually harmful, it was suggested that injurious effects on the testicle by gonad hormones were produced through inhibitory influences upon the hypophysis. Such an inhibitory effect was believed to reduce pituitary secretions to such low proportions that the testes were not maintained in normal conditions. It was pointed out that the injury from gonad hormone administration was prevented by an occasional fresh pituitary implant, hence it was suggested that gonad hormones had no direct effect upon the gonad of either sex.

The premise for this proposal rested upon several different types of experience. *a*). All proven injurious effects on gonads from gonad hormone administration, androgens or estrogens, were non-existent provided sufficient gonadotropic substance was supplied from fresh pituitary tissue or other sources. *b*). Estrogenic damage on the testicle was not repaired by androgens. *c*). Injuries to testes caused by hypophysectomy were not repaired by ordinary physiological doses of androgens. *d*). Testicular damage resulting from deficient vitamin B, or inanition, was not improved by androgens. The reciprocal relation between the gonad and hypophysis was suggested as a probable major influence in regulating reproductive conditions, at least in animals that reproduce continuously.

It is the purpose of this report to review briefly the developments in the problem of the effect of androgens on the testicle since the proposal of the above working hypothesis, and also to report new experiments on the effects of androgens on the testes of the opossum from the indifferent gonad stage to the sexually mature condition.

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Review of Studies on Effects of Androgens on the Testis

Because of the differences in the testis at different periods in ontogeny it appears of advantage to discuss three separate age periods, such as *a*), the stage of the undifferentiated gonad through early sexual differentiation; *b*), the post-natal, or juvenile stage, including early spermatozoon formation; and *c*), the period of sexual maturity. The latter period will include also the inactive stage in seasonal breeding forms, eunuchoidism, hypophysectomy, vitamin or nutritive deficiencies.

Undifferentiated gonad through sexual differentiation. Androgens have been applied to different species during the indifferent gonadal stage as well as during the period of early sex differentiation.

Among fish the testes of the minnow, *Lebistes reticulatus*, failed to undergo any perceptible modification from short periods of treatment prior to appearance of external secondary sex characters. Treatments exceeding three weeks in duration have been reported to inhibit spermatogenesis (7).

Amphibians have given various responses to androgenic treatments applied during the course of larval development. *a*). Testicular development is not affected but ovaries may be gradually transformed into testes, or testis-like organs, in *Ambystoma punctatum* (8), in *Rana temporaria* (9), and in *R. climitans* (10). *b*). Similar results were obtained with *R. catesbiana* (11), provided the androgenic treatment was accompanied by treatments with pituitary substance to hasten the normally slow development of gonads in this species. Such treatments produced 100 per cent testes. *c*). Androgens do not markedly modify genetic ovaries but they may induce feminizing changes in the testicle in which spermatogenesis is inhibited, or the testis modified in the ovarian direction; such effects appeared after androgenic treatments of *A. maculatum* and *A. tigrinum* (12), and of *A. opacum* (13).

The turtle embryo, among reptiles, developing under the influence of androgens injected into the egg during development, revealed a masculinizing influence on the ovary; the medullary portions were apparently stimulated without a marked inhibition of cortical portions (14). In the immature alligator no definite effect from androgens was noted on the ovarian medulla or testis (15).

Among birds, the chick has served most frequently for the study of effects of hormones. Androsterone, injected into the developing egg, was believed to convert all females, and some males, into a state in which the gonad was intersexual (16). Wolff's contentions received essential substantiation by Willier and coworkers (17, 18) who found that androsterone stimulated incipient cortex on the left testis and, in high doses, changed the medulla (or testis proper) into a typical ovarian medulla, thus producing an ovotestis; the right testis may also be converted into ovarian medulla, without cortex. Differing from Wolff, these investigators failed to note effects of androsterone on genetic ovaries.

Observations on mammals have been quite consistent in that chemical androgens fail to exert any modifying effect on the differentiation of the gonads in the guinea pig (19), mouse (20, 21), rat (22–25), or opossum (26–29).

Post-natal or juvenile stage. The influence of androgens on the testicle during this period in ontogeny has been studied principally on several species of birds and mammals; different androgenic preparations of concentrates, or pure chemical substances, have been employed. The results in the majority of cases have been inhibition, or injury, to the testis.

Following the original reports (1, 2) that treatments of two to three weeks with bull testis extract, introduced into young rats from birth up to 60 days of age, caused reductions in fresh testis weights of 50 to 70 per cent below that of untreated littermates, it was shown that the pure chemical hormones androsterone (30), testosterone and testosterone propionate (31) exerted entirely similar effects. Pronounced histological damage and prevention of spermatozoon formation occurred. The dosages of androgens utilized were approximately physiological doses, in the sense that the amounts injected were sufficient to maintain a growth of accessory reproductive organs in castrate rats equivalent to that induced by intact testes; in animals up to an age of 90 days, and for testosterone propionate this represents 0.15 mg. daily, or less for younger ones. When, however, daily doses were increased

up to 1.0 mg. per day the testes were less inhibited in their development. It was noted also that as animals became older a given dose caused less inhibition; at the 80 to 90-day age only slight reduction in weight of testes frequently occurred in 20 days of treatment. The explanation for these findings is not at present satisfactory. The original idea of an indirect effect through the pituitary gland does not clearly account for reduced effectiveness with higher than physiological doses at similar ages.

Reductions in testis weight have been reported for young rats by several investigators (33–40). Concerning results with other than physiological doses, considerable disagreement exists since very small doses (38) and very large ones (35, 37) are alleged by some to stimulate the testes, whereas others (40) deny stimulation of testes by large doses.

Injuries to the testes of several different species have been shown to follow androgenic treatments. Thus, young dogs experienced decreases in weight of 50 per cent (41); and the duck (42), chick (43), pigeon (44), and guinea pig (45) have revealed marked injuries after treatments with androgens. In a following section new observations on the effects of the administration of androgens to the normal male opossum during testis differentiation, juvenile and puberal stages, will be presented briefly.

Mature testes. In the normal mature rat (100 days or older) Moore and Price (2, 30, 31) failed to note marked involutionary changes in the testes as a result of androgenic treatments, presumably from a lack of sufficiently high dosage levels. However, some investigators have reported injurious effects in adults while others deny such actions; dosages up to 30 mg. daily have been employed, representing 100 or more times a physiological dose. Injuries have been reported in rats above 100 days of age receiving up to 7.5 mg. of testosterone or testosterone propionate daily (46). Androsterone applied locally on the comb of adult cocks, causing marked comb growth stimulation, led to definite testis injury (47). In man, observations of effects of androgens on the testis are few and have involved eunuchoidal states more frequently than normal ones. Some slight suggestion of improvement in testis function after treatment has been recorded (48). Lack of appreciable effect in eunuchoids (49), and definite injury revealed by a diminution, or complete absence, of spermatozoa in the ejaculates (50, 51, 52) represent largely the type of report of effects on the human testis. The lack of histological observations on testes of man after treatments with androgens may be rectified in the near future.

Special conditions. Hypophysectomy in the rat was clearly demonstrated by Smith (53) to induce immediate involutionary changes in the testis, and many subsequent investgators have confirmed this finding on several different species. In experiments carried on by Vatna (54) the testes of rats, already degenerate as a result of hypophysectomy, were not visibly repaired by androgens administered in amounts sufficient to repair involutionary damage on seminal vesicles. Walsh, Cuyler, and McCullagh (55), by injections of urinary androgens immediately following hypophysectomy in the rat, were able to prevent the usual involutionary changes in the testes. This finding was confirmed by others who employed various androgens (56–59). Thus, the rat testis was protected against involution following hypophysectomy, but was not restored to sperm formation if injections were delayed until damage had occurred. Similar protection against involution in the testis after hypophysectomy was provided by progesterone (60) and by yeast extracts (61), and the effectiveness of various androgens in protecting against these changes was not shown to be parallel to their effectiveness in stimulating accessory reproductive organs (62). In 1940, Nelson (63) noted that administration of adrenal cortex extract caused a diminution in the usually rapid involution after hypophysectomy, and when such treatments were followed by androgens the degenerate rat testis was restored to spermatozoon formation; later he (64) was able to restore spermatozoon formation by daily treatments with 3.0 mg. of testosterone propionate alone although the testes were much smaller than normal. Complete repair with androgenic treatment has not yet been attained.

Other species, likewise, have been noted to continue spermatozoon production after hypophysectomy if androgenic treatments were begun immediately following removal of the hypophysis. Thus, such protection has been reported for the mouse (65), rabbit (66), pigeon (44), ground squirrel (67), and guinea pig (68).

Some further evidence has been found to suggest a stimulating influence on the testis from androgens. Rats, hypophysectomized prior to sperm formation and treated for 15 days, were found to have completed spermatozoon formation (69). Immature ground squirrels, as well as older males during the season of testicular inactivity, responded to androgenic treatments with formation of spermatozoa (70).

Androgenic treatments failed to repair testis damage in other experimental conditions in which testis activity was in a low state, presumably because of secondary involvement of the pituitary. These conditions include injuries resulting from *a*), estrone treatments; *b*), vitamin B-deficient diet, or *c*), partial inanition. Dosages of physiological levels, such as the equivalent of activity in 0.2 mg. of testosterone propionate have been used (2), but large overdosages up to 30 mg. daily have not been utilized in this type of experiment.

Thus, this brief review of developments during the last dozen years relative to the effects of androgens on the testicle reveals many discrepancies, emphasizes many species differences in reactions to the same and to different androgenic preparations, and raises many unsolved problems. The major evidence appears to indicate *a*), that, whereas androgens may modify embryonic development of the undifferentiated gonad in some species of fish, amphibia, reptiles and birds, they do not appear to exert an appreciable influence in mammals on the undifferentiated gonad, or the differentiating testis. *b*). Predominantly, androgens appear to inhibit the growth of the testis and its spermatogenetic activity during the period of puberal development. More evidence appears to be required to substantiate a gametokinetic, or germ-cell stimulating action, with dosages much below ordinary physiological ones. *c*). The suggestion is strong that the action of androgens may prove to be somewhat different in animals that reveal strictly seasonal reproductive activities and those exhibiting a continuous reproductive pattern. *d*). Although androgens exert a protective action against testis involution following hypophysectomy, and exert some reparative action after degenerative changes have been induced, it is still a question whether this is a direct action upon the testicle, or whether the power exercised has any relation to its ordinary androgenic properties; the possibility of influences from some metabolite from the relatively large amounts of substance usually required should be kept in mind. *e*). It appears clear that androgens cannot be regarded in any general sense as testicular stimulants. *f*). The effects of androgens upon the testis remain an open question.

MATERIALS AND METHODS

The opossum was chosen for a study of the effects of androgens on the testicle during the period from the indifferent gonad, through the period of testis differentiation, and including stages sufficiently mature that the testis is producing spermatozoa and secreting hormone in sufficient amount to place the accessory reproductive organs in a functional breeding state.

Pairs of males were selected of which one was treated with testosterone or testosterone propionate[2] and the other served as untreated control. Eleven such pairs, representing 10 different periods in development, are included in table 1 with data on age, size, treatment, and results. Serial sections were available for all testes excepting the three older stages; testes of the latter were sectioned in paraffin but not completely. On *day 82*, and later, testes were sufficiently large to be dissected under a binocular microscope and weighed. The size of the testis from *day 16* to *day 50* did not permit weighing and comparisons between the treated and normal control were made possible by projection of each fourth or fifth section of the testis

[2] The hormones employed were provided through the courtesy of Dr. G. Stragnell of the Schering Corp., Bloomfield, N. J.

at a magnification of 98 diameters followed by planimeter readings, and conversions to account for all sections of a testis; final recorded planimeter readings were the averages of three determinations for each organ, in which variations were less than one-half of one per cent. Standardization of planimeter measurements for all testes of the younger group renders them readily comparable. Testis weights were available for the six older stages.

Androgen was applied to the treated member of the youngest pair (*days 3–15*) by ointment containing testosterone, spread entirely over the body daily by means of a small artist brush; all others were treated by daily subcutaneous injections

TABLE 1. DATA FOR PAIRS OF OPOSSUM POUCH YOUNG AT DIFFERENT AGES, NORMAL AND ANDROGEN TREATED

Animal	Age, Days[1]	Snout-Rump Length, mm.	Head Length, mm.	Weight, gm.	Phallus, mm.	Treatment, Days; mg.	Testis Size, Planimeter Units or Weight in mg.	Prostate Weight, mg.	Average Diameter 25 Sem. Tubules mm.
33A	K 16	33.0	10.7	1.35	1.5X1.0	Normal control	13,640 pl. u		.060
96A	K 16	36.3	12.5	1.87	2.2X1.2	3–15; T oint.	19,160 pl. u		.060
34B	E 32	54.0	19.0	5.67	1.5X1.2	Normal control	405,930 pl. u		.067
30A	E 32	54.0	17.5	5.83	3.0X2.2	16–31; TP,[2] 0.25	418,430 pl. u		.079
87A	K 44	65.5	23.4	10.00	1.8X1.5	Normal control	1,019,625 pl. u		.078
221	K 40	64.4	21.9	8.23	2.3X2.0	30–49; TP, 0.25	1,019,385 pl. u		.078
41	E 52	80.0	27.4	15.03	1.5X1.5	Normal control	2,020,275 pl. u		.073
223	K 49	77.8	25.6	11.84	4.3X3.2	30–48; TP, 0.25	1,531,290 pl. u		.072
91	E 83	135.0	46.0	72.0	4.4X2.4	Normal control	2 testes, 43.6 mg.		.075
92	E 83	136.0	44.5	72.0	7.5X3.5	70–82; TP, 0.5	2 testes, 49.6 mg.		.078
212	E 93	176.0	56.9	144.0		Normal control	2 testes, 81.6 mg.		.067
214	E 93	175.0	54.4	116.0		80–93; TP, 1.0	2 testes, 86.0 mg		.075
82A	E 100	200.0	63.0	180.0		Normal control	2 testes, 145.6 mg.		.076
98	E 100	200.0	58.0	168.0		70–99; TP, 0.5	2 testes, 140.0 mg.		.078
176	E 5 mo.			370.0		Normal control	2 testes, 513.8 mg.	107	.127
177	E 5 mo.			345.0		Normal control	2 testes, 464.0 mg.	94	.130
172	E 5 mo.			290.0		13 days; TP, 2.0	2 testes, 369.8 mg.	780	.127
173	E 5 mo.			260.0		13 days; TP, 2.0	2 testes, 472.4 mg.	858	.127
68	E 8 mo.			906.0		Normal control	2 testes, 3100.0 mg.	1920	.230
67	E 8 mo.			1011.0		15 days; TP, 5.0	2 testes, 3730.0 mg.	3900	.230
72	E 10 mo.			3737.0		Normal control	2 testes, 2509.0 mg.	4060	.250
73	E 10 mo.			4643.0		33 days; TP, 5.0	2 testes, 2400.0 mg.	4340	.240

[1] K, known age; E, estimated age.
[2] TP, testosterone propionate.

of testosterone propionate in sesame oil. The effectiveness of such treatments on the opossum for androgens and estrogens has been described earlier (29).

EXPERIMENTAL RESULTS

Day 16. The youngest opossum to receive treatment with androgens was autopsied on the 16th day after entering the pouch; the gonad can be identified as a testis by *day 5*. Although dosages in ointment cannot be accurately stated the effectiveness of the treatment can be judged from responding structures.

At 16 days of age the testis is attached by mesentery to the mesonephros which reveals beginning degeneration in the anterior end; this organ is an active excretory organ until about *day 14*. The Wolffian duct responded to some extent to the androgenic treatment but it was pointed out previously that this duct responds only moderately to androgenic treatment. The prostate gland shows marked responses, however, and from the data in table 1 it can be seen that phallic response was definite.

The testicle at this age has a definite tunica albuginea in the process of formation. Solid cords of cells represent future seminiferous tubules which are distributed in a

peripheral position with the central portion of the organ consisting of unorganized mesenchymal cells. These solid cords possess a basement membrane, and ocular micrometer measurements of cross sections of the cords show them to vary between .05 and .07 mm. Histological study of the testes failed to reveal noticeable differences between the treated and control organ. Projections of sections and measurements with the planimeter, of both the treated and control testis, were made in order to

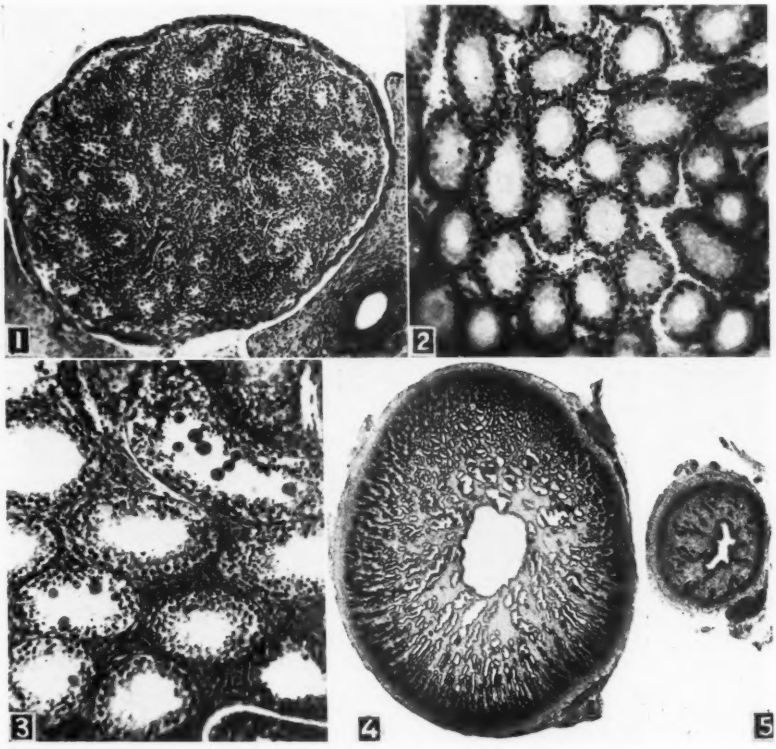

Fig. 1. SECTION OF TESTIS from androgen treated, 32-day-old opossum (*Animal* 30A; testosterone propionate, 0.25 mg. daily, *days* 16–31): Wolffian duct (vas deferens) lower right. ×60. Fig. 2. SEMINIFEROUS TUBULES from testis of normal opossum. 5 mo. of age. (*Animal* 177). ×60. Fig. 3. SEMINIFEROUS TUBULES from testis of androgen treated opossum, 8 mo. of age (*Animal* 67; 15 daily injections testosterone propionate, 5.0 mg. daily). Tubules show degenerating giant cells. ×60 Fig. 4. CROSS SECTION OF PROSTATE GLAND from androgen treated, 83-day-old oppossum (*Animal*. 92; testosterone propionate, 0.5 mg. daily, *days* 70–82). ×15. Fig. 5. CROSS SECTION OF PROSTATE GLAND from normal, 83-day-old opossum (*Animal* 91). ×15.

compare volumes of the two; measurements are recorded in table 1. On the basis of planimeter units the treated testis showed a 40 per cent increase in volume.

In this pair, therefore, testosterone treatment appeared to induce a greater volume but did not cause perceptible histological differences.

Day 32. *Animal* 30A, receiving daily injections of 0.25 mg. of testosterone propionate from *day* 16 to *day* 31, was compared with the normal 32-day control. Prostate

response was marked, since the total diameter of the urogenital sinus region was double that in the control, and prostate outgrowths emphasize, even more, the tremendous stimulation imparted to this structure. Wolffian ducts responded also since the greatest luminal diameter of the control duct was .037 mm. in comparison with the treated duct lumen of .097 mm.; the vas deferens approaching the genital cord in the normal possessed a lumen of .007 mm. and the corresponding portion of the treated vas deferens was .06 mm.

A comparison of the testes of the treated and control male failed to reveal any histological difference between the two. The condition of the seminiferous cords was entirely similar and table 1 shows average diameters to be similar. Total planimeter volumes show the treated testis to be but 3 per cent above the control. A section of the testis from the treated animal can be seen in figure 1.

Day 40. The 40-day-old treated male was compared with a normal male of *day* 44; the treatment induced marked responses in the prostate, vas deferens (100 per cent increase in vas lumen diameter), and a fairly marked response of the phallus.

Histological study of the normal and treated testis failed to make evident any detectable differences. In each case the average diameter of 25 tubules was the same. Comparative planimeter determinations of testis volume indicate that the treated testis was a fraction of one per cent below that of the normal testis in the control animal.

Day 50. In this pair of males the 49-day-old treated one was compared with a 52-day normal. The treatment with androgens evoked marked responses in the prostate gland, Wolffian duct, and phallus.

The testes of the two males did not present any differences. Histologically the treated and control were entirely similar; tubular diameters were similar. Planimetric determinations indicate a 25 per cent decrease in volume of the androgen-treated testis. One notes a consistent downward trend in the treated testis from a 40 per cent advantage at *day* 16, to 3 per cent on *day* 32, an essential equality on *day* 40, to a 25 per cent disadvantage on *day* 50. Whether this trend is of any significance is uncertain, but in any event no detectable difference in histology or tubule size was noted at these four ages.

Day 83. At this age the testes can be dissected, and weighed, and for these six older stages weights for this organ are available.

The androgenic treatment with 0.5 mg. of testosterone propionate daily on *days* 70 to 82 induced an enormous response on the part of prostate gland (compare fig. 4 and 5), vas deferens, and phallus. The weight of the entire reproductive tract in the normal control was 114 mg. as compared with the weight in the treated male of 464 mg.; thus accessory reproductive gland response to the hormone treatment was pronounced. The greater testis weight in the treated male (approximately 13 per cent) is believed to represent, in the main, a response of the epididymis attached to the testis.

Histological study again revealed no detectable difference between the normal and treated testis, and diameters of the seminiferous cords were the same.

Day 93. In the 93-day-old pair of males 1.0 mg. of testosterone propionate daily for 14 days induced a weight of the reproductive tract of 840 mg. as compared with the normal of 500 mg. Prostate response was marked, since in the normal organ the cord outgrowths from the urogenital sinus epithelium were still solid, but branched, whereas in the treated gland, outgrowths were fully developed and canalized to the peripheral extension of the cords, making a prostate typical of a normal breeding state.

Weights of the testes were 81.6 mg. for the normal and 86.0 mg. for the treated; the slight increase in weight of the treated organ apparently represents a response of the epididymis. Histologically the testes were entirely similar; seminiferous cords were solid, largely quiescent in mitosis, and relatively compact with but little ordinary appearing connective tissue cells between the tubules. Average diameters of seminiferous cords were quite similar.

Day 100. The effectiveness of the androgenic treatment is seen by comparing the weights of the entire normal reproductive tract of 265 mg. with that of the treated male of 1930 mg. Testis weights of 145 mg. for the normal and 140 mg. for the treated are in keeping with the histology which also failed to reveal any recognizable difference in the two organs. Tubule diameters (.076 normal, .078 treated) not only failed to show a difference between the treated and control testis, but at the same time they show that no tubular activity had occurred since about *day* 32. By planimetric measurements, it is indicated that the normal testes increased more than 100 times in volume from *day* 16 to *day* 50; and by weights, normal testes between *days* 83 and 100 increased approximately 4 times, yet from *day* 16 to 100 tubular diameters had not increased. Testicular growth apparently had involved only increases in length of seminiferous tubules. Spermatogenesis had not begun.

Five months of age. The stage at 5 months of age is represented by four males, two of which were controls while two received identical treatments with 2.0 mg. of testosterone propionate for 13 days preceding autopsy. The average weight of testes for the two normal males (weights for each animal recorded in table 1) was 489 mg. and for the treated males 421 mg.; the epididymis of this and later stages is not included. Average prostate gland weights for the two normal males was 100 mg. in comparison with 819 mg. for treated males; thus the treatment induced a marked stimulation of the prostate.

The testes at this stage are decidedly different from younger organs, particularly in that the seminiferous tubules contain a definite lumen (see fig. 2). The majority of the tubules possess a single layer of peripherally located cells and, in places, two layers of cells now constitute the actively developing germinal epithelium; mitosis is more marked. Intertubular areas contain apparently ordinary connective tissue cells, as well as a few cells assuming a more glandular character with pronounced cytoplasm; these are the Leydig cells.

The treated testes did not differ from normal ones either in tubular content, tubular diameter, or character of intertubular tissue. Whereas the average weight of treated testes was somewhat lower than in normals it will be noted that the total body weight was also lower, and the reduced testis weight cannot be taken seriously for a direct effect from the androgen.

Eight months of age. The treated male of this pair received 5.0 mg. of testosterone propionate for the preceding 15 days. Prostate weight in the normal (1.92 gm.) and the treated (3.90 gm.) showed a smaller relative response to treatment than in the preceding stages, due apparently to the awakening of hormone secretion in the normal male. The control prostate at this stage is developed to full breeding condition with glands canalized to their peripheral ends; this would represent greater development than that shown in figure 4. The histological condition of the normal and treated gland is quite similar since additional hormone in mature development may add to, but not change, the histological character of the gland.

Spermatozoa are now present and all stages of spermatogenesis can be noted; the testis is essentially mature although differing in one respect from older stages. At the luminal edge of the germinal epithelium, and frequently within its midst,

the normal testis tubules contain some scattered, discrete masses of fused cytoplasm and nuclei representing the characteristic 'giant cells' of testis degeneration. While not numerous these giant cells are conspicuous and may represent a lack of maturity similar to involuting follicles in an ovary at puberty. It appeared that the number of these giant cell masses was greater in the treated testis than in the normal but if the apparent difference is real it represents the only difference between the treated and normal testis. Figure 3 shows a few tubules from the treated testis containing these masses in the lumen or in the germinal epithelium.

Testis weight (normal 3.10 gm.; treated, 3.73 gm.) showed a slight increase in the treated animal, which is also somewhat heavier in total body weight. Average tubular diameter was the same in the treated and control testis (.230 mm.) and the number of tubules showing spermatozoa was similar in both. Intertubular tissue was not apparently different in the control and treated animal.

Ten months of age. The two animals of this age were not born in our pens but were received in shipment from Kentucky. Condition of the animals, their general development, total body weight, and condition of the reproductive system suggests that they were older than our own pen-reared animals, but still within the year of their birth. In all probability they were born during early February (a few litters are born in Kentucky in late January) and these were autopsied in December; they are estimated to be 10 months old at autopsy.

The treated male received 33 daily injections of 5.0 mg. each of testosterone propionate prior to autopsy. The normal prostate weight of 4.06 gm. and the treated, 4.34 gm. represents only a small increase in size, but the animals were in a full breeding state. Testis weights were similar.

Histologically the testes of control and treated males were entirely similar. All tubules were in active spermatogenesis with quantities of spermatozoa, and the epididymides contained motile germ cells. Intertubular areas, now showing large amounts of interstitial cells, are not perceptibily different; tubular diameters are similar. In this case, as in all others, no definite effects on the testis can be seen from the androgenic treatments.

SUMMARY AND CONCLUSIONS

A summarizing statement on previous findings relative to effects of androgens on the testicle is given.

In the opossum the testis increases markedly from the 16th to 60th day after birth and more slowly from *day 83* to maturity. Seminiferous tubules are quiescent up to *day 100* and although apparently increasing in length they do not increase in caliber from *day 16* to *100*. Activity begins by the 5th month of age; testis weight increases; tubules acquire a lumen; mitoses are numerous; Leydig cells are apparent, and the prostate gland shows decided hormonal stimulation. Spermatozoa are present in the tubules by the 8th month.

Androgenic treatment during ten different periods from birth to maturity failed to produce consistent modifications in the testis; slight volume changes at early periods appear to be of questionable significance.

In the absence of some consistent response it is concluded that androgenic treatments have failed to show any consistent effect on the testicle between birth and maturity in the opossum.

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THE CLINICAL ASPECTS OF DWARFING

E. KOST SHELTON

HARRIS (1) in his introduction to Volume II of the Cold Spring Harbor Symposia on Quantitative Biology writes as follows.

> As we proceed from these relatively simple, single-celled organisms to multicellular animals, including man, the phenomenon of growth becomes almost indescribably complex. The environment of a given cell comes to include other cells contiguous to it, or remotely placed in the same organism. Functional, mechanical and nutritive conditions, dynamic interaction between cells and parts, 'organizers,' endocrine secretions, these and other variables are added to the cellular environment within the organism itself. Genetic factors are continually in the background from the moment of the formation of the germ cells, and at least some of these factors are always reacting with both the internal and external cellular environment.

Such is the subject I have been asked to discuss and it is with considerable trepidation that I approach the task even from the comparatively secure vantage point of my own experience. The discussion is to be clinical but there are several philosophical concepts regarding growth and its usual concomitant development to be disposed of at the outset. According to Wetzel (2):

> Growth is accomplished fundamentally by the process of cellular self-multiplication and it is, therefore, basically concerned with change in size, that is, with change in cell number, length, area, volume, or mass. Differentiation, meanwhile, effects a change in structure. From the phylogenetic as well as from the ontogenetic point of view, growth is certainly the more primitive attribute, differentiation the more recent as well as the more highly specialized acquisition of organisms. But, in contributing to the development of a single being, growth and differentiation act in conjunction. This implies organization. The living result, an organism, thus owes an indeterminate share of its final arrangement to each of these processes; yet we may say that it attains size by virtue of growth, and architectural style by the concomitant process of differentiation.

Wetzel's remarks are exceptionally apt. By his method of reasoning, development is a natural concomitant of growth, and vice versa, but, however intimately related, each is entirely distinguishable. As an explicit example, Carrell's potentially immortal chicken heart cells grow continuously in an adequate environment but never develop. The predestinator of order, that something which would make them a heart instead of a mass of useless organic matter, is absent. Growth appears to be a wild, functionless, chaotic Frankenstein moving in an aimless fashion toward an endless goal. Development on the other hand is that unknown spark of cell intelligence which guides its destiny, whips growth into order and ends in the consummation of a preconceived plan. Both are inherent in the cell and when taken with the reproductive and survival mechanisms constitute that strange phenomenon called life.

Todd (3) speaks of development as maturation, or as the maturation process, to wit:

> Disturbed progress in maturity shows itself only secondarily, if at all, in stunted growth; it may be accompanied by excessive stature, for dimensional growth has its own controlling factors which operate separately from, though normally in harmony with, those which control the maturation process. One would not indeed expect to find a human being where growth and maturation have entirely divorced from each other. There are examples however in which inhibition of growth greatly predominates.

Although Todd more than any other touches upon the clinical aspect of this subject and I thoroughly agree with this concept, his terminology appears unfortunate. To me the word maturation connotes the final stage or fulfillment of development rather than the process itself and the disctionaries, apparently, bear me out.

It may seem like splitting hairs to dwell upon the differences between growth and embryonal unfolding (development) but the subject has very important clinical significance especially in endocrinology. Many able observers have made scholarly contributions to this subject but unfortunately few clinicians, even those handling children every day, appear conversant with the matter.

Well-grown but poorly developed children and poorly grown, well-developed children are not uncommon. Because of the physiological timing of certain structural phenomena, one sees evidence of such inequality throughout life. To use simple examples, consider the somatic destinies of brothers, one of whom develops macrogenitosomia and the other suffers traumatic castration at the same early age. With inherently equal growth potentials, the one is bound to be short and the other tall, while the variations of body development are certain but unpredictable. Or, observe the classic cretin, wherein growth, while retarded, attempts to struggle through an arrested and wholly inadequate structural unfolding. The ultimate product is as incongruous as a house would be if the plasterer came before the plumber or the bricklayer worked continuously without regard for the carpenter or his other fellow artisans.

Considerable speculation has arisen as to the chief glandular exponents of these two processes and Todd makes the following statement:

> The maturation process, like growth, is a complex phenomenon and, therefore, like growth, depends for its full expression upon general constitutional fitness rather than upon the influence of a single controlling factor. If anything, thyroid is dominant in growth promotion and anterior lobe hypophysis in maturation.

I am not in accord with these views but am more than ever convinced there is ample evidence to show that the thyroid is more concerned with the process of embryonal unfolding than of growth, and that the opposite may be said of the pituitary. In hypophysectomized animals (without concomitant cerebral damage) one sees a small but fairly well-developed organism. It is true that certain structures, as the teeth, the sinuses, even the bony skeleton, do not differentiate at the proper time and some, such as the gonads, rarely do develop. On the other hand, in spite of the diminutive stature, the finished product is far from remarkable. It is also conceded by most observers that hypothyroid dwarfs show a much greater degree of osseous retardation than hypopituitary dwarfs of the same chronological age, as evidenced by roentgenograms of the skeleton (4, 5, 6). The development of the central nervous system is further proof of this contention. Hypophysectomized animals frequently reach a degree of intelligence, as measured by their behavior, not far behind the normal (7). Pituitary dwarfs have fairly normal (some excellent) intelligence, although their sexual inadequacy and absence of normal sexual behavior, makes them appear retarded and childlike (8–9). We are all aware of the pituitary-thyroid relationship. Remove the pituitary and one produces a degree of secondary thyroid atrophy and dysfunction (10–11). Aside from the sexual dystrophy, which is unquestionably a pituitary phenomenon, most of the developmental retardation could and probably does result from secondary thyroid inadequacy. In fact, there is evidence to show that the thyroid hormone, when administered in conjunction with anterior pituitary preparations, will enhance tissue differentiation in hypophysectomized animals (12) and in pituitary dwarfs (13).

On the other hand, consider the thyroidless individual. Even in the presence of

an intact pituitary ones sees a badly distorted gnome-like creature with umbilical hernia, poorly differentiated muscular and osseous structure, and an embryonal nervous system. It is true, cretins are nearly always dwarfed. The growth inadequacy may rest partly with the thyroid *per se* but it is easy to believe that the pituitary is no wise less embarrassed than the other body economy and fails to exert its function so far as the growth sequence is concerned.

A number of years ago I attempted to elucidate the problem of growth deficiencies in children by developing a classification based upon the etiologic factors involved. The work was prompted by my own confusion regarding this matter, but was never published. Experience has convinced me of its practical working value. Perhaps others may profit by a better understanding of the several factors involved, for while no doubt most clinicians take all of these factors into consideration, nothing is quite so confusing as disorganization unless it is disorganization plus a chaotic terminology. Allow me first to submit the types of dwarfism and infantilism as classified in Dorland (14). The italcized numbers in parentheses refer to the numbers used in my own classification.

I. Dwarf: an abnormally undersized person. *Achondroplastic.* A dwarf showing short extremities, trunk of normal size, large globular head, stumpy nose and strident hands. Such dwarfs represent a dwarfed adult condition of the skeleton. (2). *Asexual.* An adult dwarf with deficient sexual development. (4C). *Ateliotic.* A dwarf whose skeleton is infantile in condition with persistent non-union between epiphyses and diaphyses. (2, 4A-B-C). *Brissaud's.* Infantile myxedema. (4C). *Deformed.* A person who is both abnormally undersized and deformed. (2-3-4C). *Infantile.* A person with marked retardation of mental and physical development. (2-3-4A-B-C-5). *Micromelic.* A dwarf with very small limbs. (2-1). *Normal.* A person who is merely undersized but not deformed. (1-3-4A-B-C-5). *Phocomelic.* A person in whom the diaphyses of the long bones are abnormally short. (2). *Physiologic.* A normal dwarf. (1-2-3-4A-B-C-5). *Precocious.* Retardation of growth but acceleration of development of the young resulting from feeding pineal extract to the parents and forebears for several generations. (4C). *Primordial.* A normal dwarf. (1). *Pure.* A person of small stature but well formed (1-3-4A-B-C-5), *Rachitic.* A person dwarfed by rickets, having a high forehead with prominent bosses, bent long bones and Harrison's sulcus. (4A-B). *Sexual.* A dwarf with normal sex development. (1).

II. *Infantilism.* A condition in which the characters of childhood persist in adult life. It is marked by mental retardation, underdevelopment of the sexual organs, and often but not always, by dwarfness of stature. *Angioplastic.* Infantilism attributed to defective development of the vascular system. (3). *Bilirubin.* Masses of bilirubin in the pyramids of the kidneys, especially in newborn infants.(3). *Brissaud's.* Infantile myxedema. (4C). *Cachectic.* Infantilism due to chronic infection or poisoning. (5-4B). *Celiac.* Infantilism resulting from celiac disease. (4B). *Dysthyroidal.* Infantilism due to defective thyroid activity. 4(C). *Hepatic.* Infantilism associated with hepatic cirrhosis. (3-4A-B-5). *Herter's.* Intestinal infantilism. (4A-B). *Hypophyseal.* A type of dwarfism, with retention of infantile characteristics, due to undersecretion of the growth-promoting elements in the hypophysis. (4C). *Idiopathic.* A form of arrested development of unknown causation. *Intestinal.* A diarrheal disease of young children but sometimes occurring in adults, tending to be accompanied by failure of growth and marked by large pale stools containing much fat. Called also 'Gee's disease,' 'non-tropical sprue' and 'idiopathic steatorrhea.' (4-A-B). *Lorain's.* Arrested physical development of unknown cause; ateleiosis. (3-4-A-B-C-5). *Lymphatic.* Infantilism associated with lymphatism. (3). *Myxedematous.* Creatinism. 4(C). *Pancreatic.* Infantilism that is marked by defective pancreatic action. (4B). *Partial.* Arrested development of a single part of tissue. (1-2-5). *Renal.* Infantilism due to disorder of the renal function. Many cases show extensive bone changes and this condition is called renal rickets or pseudo-rickets. (3). *Regressive-Reversive.* Infantilism beginning after bodily growth has been completed. (4-A-B-C-5). *Sex.* The continuance of the prepubertal sex characters and behavior beyond the usual age of puberty. (4-C-5). *Static.* A state occurring in children characterized by hypotonia of the trunk muscle and hypertonia of the muscles of the extremities. (3-4A-B-C-5). *Symptomatic.* Infantilism due to general defective development of tissue. (1-3-4A-B-C). *Tardy.* Reversive infantilism. (4-A-B-C-5). *Universal.* General dwarfishness in stature with absence of secondary sexual characteristics. (4-A-B-C).

Is this not as formidable an array of confusing and overlapping data as ever cluttered up a dictionary? One may see shortly how easy it is to classify all of the above under a few simple headings which not only make the subject more comprehensive but which also give the key to diagnosis and treatment.

If one were building a house instead of a human being he would outline his problem and his success or failure would depend very largely upon first, the plan; second,

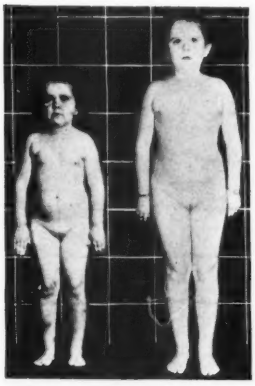


Fig. 1. J.M. PITUITARY DWARF (8, 22). Received in all 3639 cc. (approximately 23 cc. a week) of anterior pituitary extracts during a period of 3 years. Except for one very brief interlude no other treatment was given. This is the only patient that I am convinced grew as a result of the administration of anterior pituitary extracts. The patient lives in Honolulu and recent futile attempts have been made to get her present height and weight. I understand she is still very tiny although a full fledged stenographer. Present age about twenty years.

the contractor; third, the material; fourth, the proper transportation and distribution of material; fifth, the artisans who use the material and sixth, labor strikes, termites, and so-called acts of God. The construction of a human body, although somewhat more complex and automatic (not involving an F.H.A. loan) is very similar. First, one must consider the germ cell; second, the maternal environment; third, the food mineral and vitamin intake; fourth, the absorption and proper distribution of this

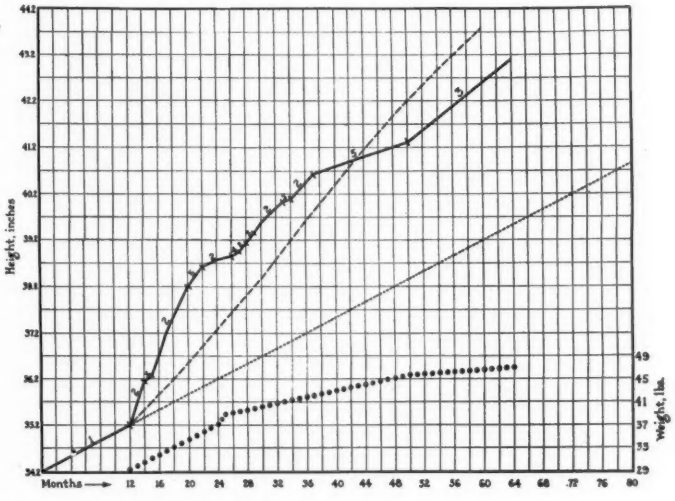


Fig. 2. J.M. GROWTH CURVE. *Solid line*, actual growth. *Dotted line*, previous expectancy. *Broken line*, approximately normal growth curve for age. Heavy dots, *weight*.

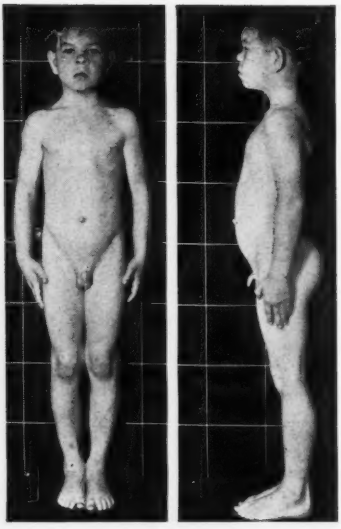


Fig. 3. *E.H.* PITUITARY DWARF, *age* 10 yr. Ht., 47 in. Wt., 47 lb. Presenting many hypothyroid symptoms. Refractory to all treatment (thyroid and anterior pituitary separately and in combination, vitamins and minerals) over a two-year period. The annual linear increment has continued at about 50 per cent of the normal without treatment. Is now 15 yr. of age, 53 in. tall, weighs 76 lb. He is in the 8th grade at school; is an attendance problem. Parent refused to allow him to be treated with testosterone.

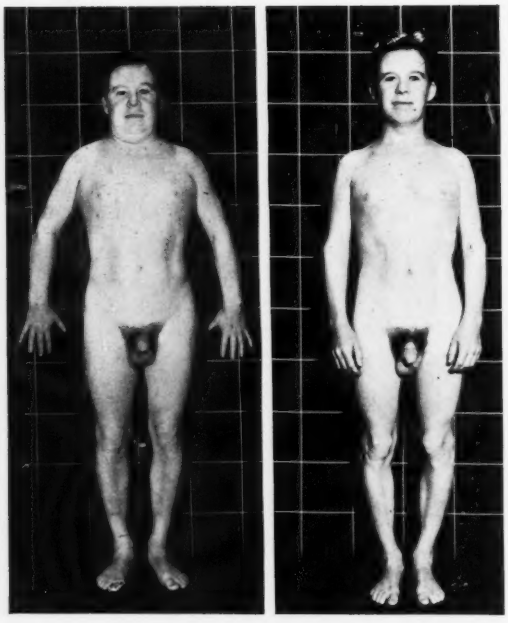


Fig. 4. UNTREATED CHILDHOOD HYPOTHYROIDISM, age, 29 yr. Ht., 60 in. Wt. 122 lb. College graduate (chemist). Evidently had suffered from childhood hypothyroidism (juvenile myxedema) since early age. Mother said always, strange, pale, silent and taciturn. No romances. Mother and father both had very high I.Q. Four months after thyroid administration (right) patient lost 15 lb. of myxedematous infiltration; did not look like same person; apparently grew almost an inch; improved markedly in social outlook.

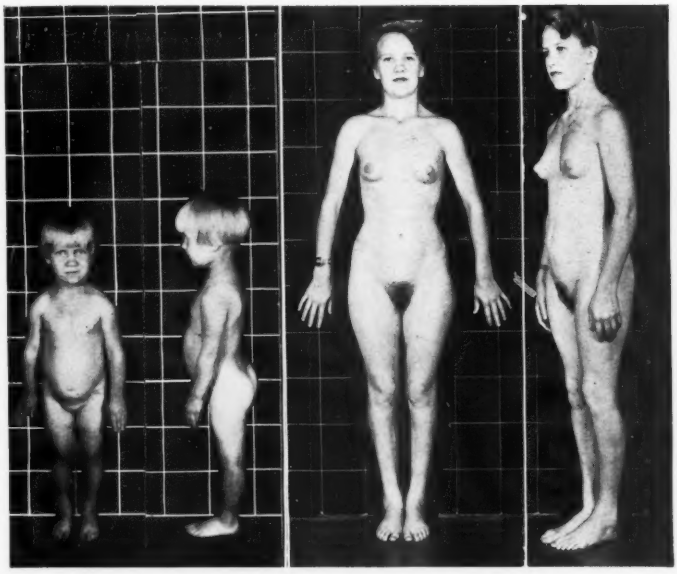


Fig. 5. *J.S.* JUVENILE HYPOTHYROIDISM, (16). Patient when first examined, 18 yr. Continuous thyroid dosage of between 1 and 2 grains daily. Catamenia normal. Is in second year high school.

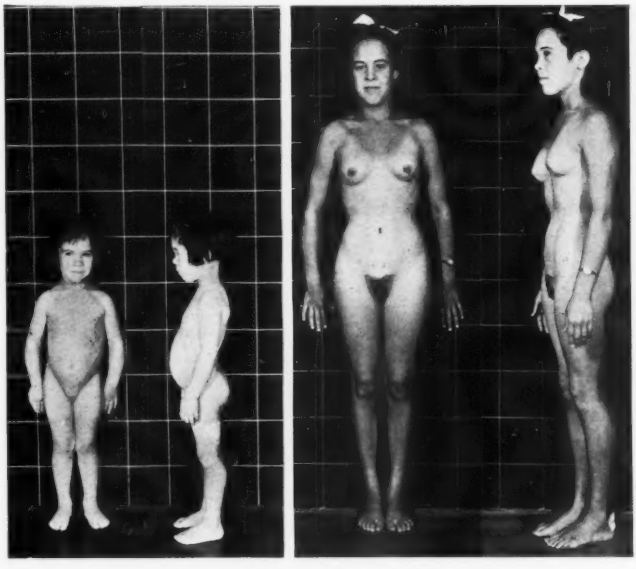


Fig. 6. *A.O.* PREVIOUSLY UNREPORTED HYPOTHYROID CHILD (author's case). Age 7 yr. 2 mos (*left*). Bone age between 1 and 2 yr. Pale infiltrated skin, mentally retarded, constipated and inactive. At age 17 (*right*). Catamenia normal. Repeated 3d and 4th grades in school but passed every grade since. Is now beginning high school.

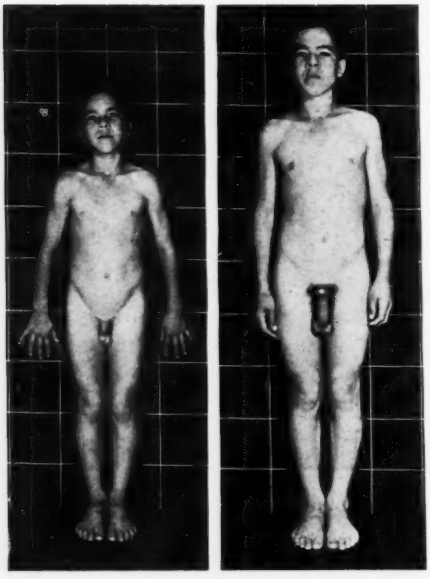


Fig. 7. *F.O.*, (22). Showing effect of administration of anterior pituitary extract and chorionic gonadotrophin (combined) on somatic growth and genital development.

material; fifth, the enzymal and endocrine systems; and sixth, disorder, disease and destruction.

With this understanding, let us go directly to a classification of growth deficiencies based upon the etiologic factors as follows:

Classification of Dwarfism and Infantilism Based upon Etiology

1. *Inherent or constitutional factors* (as in so-called primordial dwarfism and in normal small-statured persons).
2. *Congenital disturbances of the skeleton* (as in achondroplasia, mongolism and micromelic dwarfism).
3. *Anomalies of the circulatory and urinary systems* (as in congenital heart and kidney disease, angioplastic infantilism and renal rickets).
4. *Disturbances of nutrition*
   - A. Inadequate food, vitamin and mineral intake (as in slow starvation, rickets and other deficiency diseases).
   - B. Inadequate absorption of the building essentials from disturbance of the gastric, intestinal and pancreatic enzymes (as in hypochlorhydria, celiac disease, refractory rickets, intestinal nematodes).
   - C. Inadequate utilization or deposition of the essential elements from various metabolic and endocrine disorders (as in hypothyroidism, hypopituitarism, diabetes).
5. *Chronic infectious disorders* (as in tuberculosis and lues).

In some instances these factors dovetail a bit but no matter how complicated the mechanism ultimately becomes, there usually is or has been a single etiologic factor. For example, thyroid dwarfs have poor appetites and eat little, but no amount of stuffing with vitamins or minerals will enhance their growth. When given a better

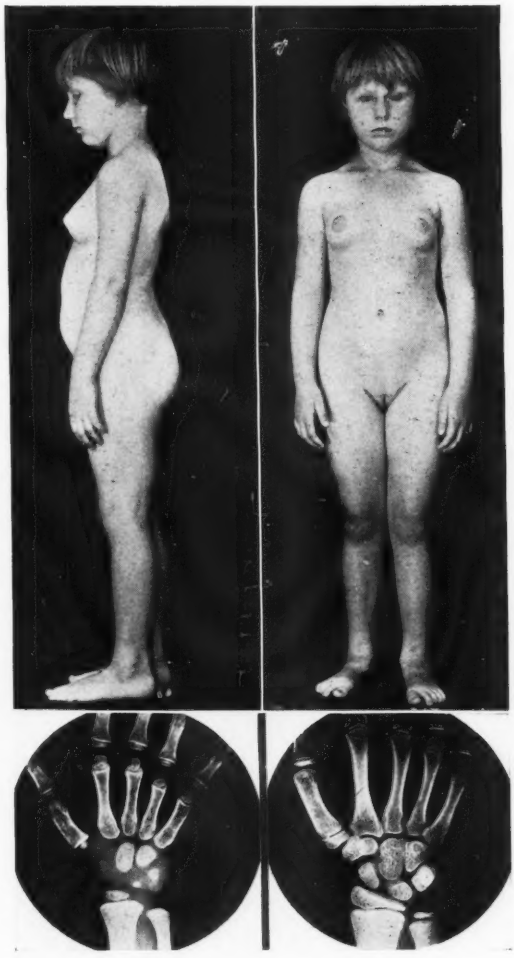


Fig. 8. PUBERTAS PRAECOX (43). 4 yr old. Regular menses since 4 mo. of age. Early and continuous elaboration of estrogens. Apparent early closure of epiphyses. Average normal bone age (*left*). Patient's bone age (*right*). This is an advance of approximately 6 years. See text.

physiological status, as by the administration of thyroid, the appetite improves; they not only ingest more food but in some manner begin to utilize it and growth is resumed at a more normal rate. The same may be said of stunted diabetic children when insulin is administered, or of children relieved from parasitic invasion of the intestinal tract.

At the outset, then, the elucidation of most types of dwarfing demands a careful and comprehensive examination. The outcome of the case may depend upon as simple a matter as the gastric acidity, a stool examination, the glucose tolerance and/or an understanding of the child's psychological pattern. Certain types such as achon-

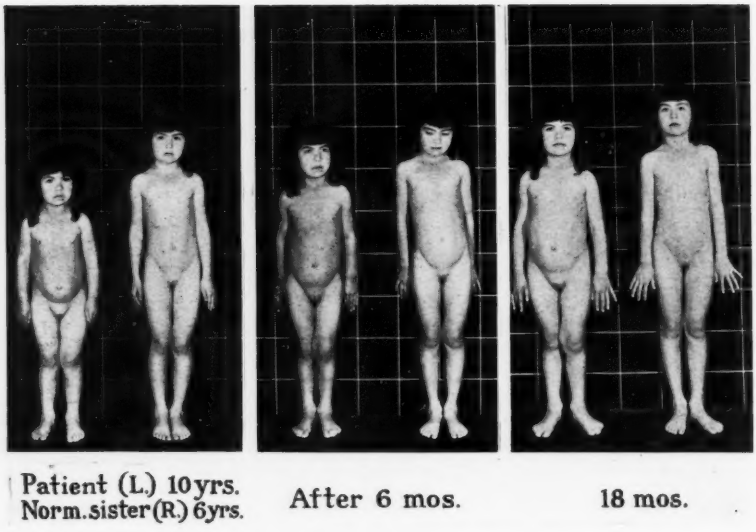


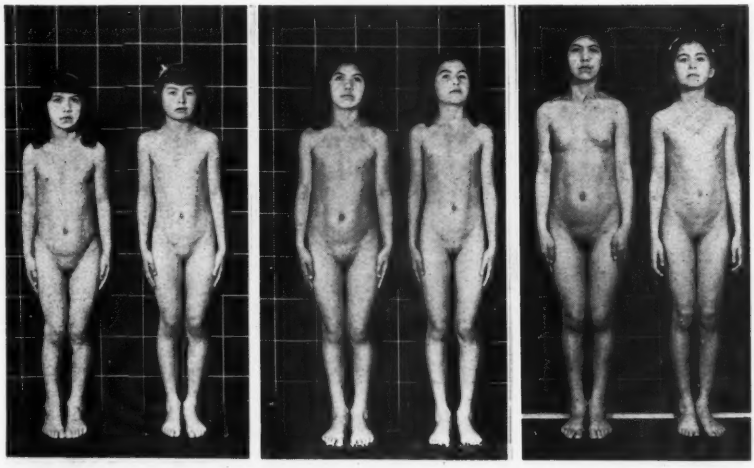
Fig. 9. C.R. HYPOTHYROID, age 10 yr. (*left*). Normal sister, age 6 yr. (*right*). Author's previously unreported case of childhood hypothyroidism. In the original photograph (*left*) the patient is 10 yr. of age, while her normal sister (*right*) is 6 yr. of age. In the subsequent photographs taken during the administration of thyroid (1–1½ grains daily) at 9 mo., 18 mo., 3, 4, 5 yr. the patient is always on the left and the untreated normal sister on the right. The patient grew several hundred percent over the normal rate for the first year or two and then gradually settled down to a normal annual increment. Note that when she reached adolescence she shot ahead of her younger sister. The intelligence improved

droplasia (chondrodystrophy) and micromelic dwarfism are easily and early discernible but many growth deficiencies escape notice until the condition is far advanced.

Space does not permit a lengthy discussion of all of the etiologic factors producing dwarfing. Those factors of shortness inherent in the germ cell as I have said, are influenced only slightly, if at all, by any form of treatment. Perhaps a potent pituitary growth factor would, if given in sufficient quantity, overcome this fixed pattern but so far it has appeared futile. One must be careful, however, not to overlook true dwarfing in the offspring of short parents, a mistake I have made on at least one occasion.

Aside from the correction of possible concomitant nutritional deficiencies in dwarfs from congenital disturbances of the skeleton, nothing can be accomplished. It is not only scientifically futile but dishonest to treat them year in and year out with endocrine products. The same may be said for dwarfing due to circulatory disturbances (mostly congenital) and so-called renal rickets. In this type, however, the general and nutritional care of the child is very important and may conceivably prevent much greater inroads into the patient's linear increment.

In the fourth classification, those children dwarfing because of nutritional and/or endocrine disturbances, one can expect to derive the most satisfactory result. You will notice I have classified the endocrine factors under the primary heading of disturbances of nutrition and under the sub-heading of inadequate utilization or deposition of the essential element for growth and development. The active principles of the endocrine glands are the utilizers of foods, chemicals and vitamins and cannot be



3 yrs. 4 yrs. 5 yrs.

markedly but a hypothyroid or myxedematous child of 10 years who has never received thyroid has in most instances suffered permanent damage to the central nervous system. This patient managed to keep abreast of her normal sister in school after 2 yr. on thyroid treatment. In other words, she was about 4 years retarded when I last saw her. The mental status may, and probably has, improved. I believe the difference in appearance of the two girls is of genetic origin rather than due to the thyroid. To observe the rapidity with which the patient's osseous system unfolded in the early months of treatment see figure 10.

separated from this important function. If all of the other factors can be excluded (and this is sometimes difficult) in a child who for a year or longer has grown but a fraction of the normal increment, one has a right to suspect some endocrine dysfunction. I shall not bore you with a description of the classic case. Anyone can make some kind of diagnosis in a child who is already a dwarf, but to detect and correct such a condition before there are marked and permanent stigmata is another matter. In the first place, one cannot be sure he is dealing with a true growth deficiency until a period of months or, in some instances, years have elapsed, unless careful height records for the previous years are available. Even then one must take the variabilities of normal growth during the epochs of life into consideration. The statements of parents are frequently misleading. Once satisfied that such a condition exists, however, one should attempt to ascertain the etiology and, if amenable, outline the treatment as speedily as possible.

At this point I should like to discuss a phenomenon which several individuals (15–16) have pointed out but which no one (to my knowledge) has adequately explained. It may be one of the keys to the entire growth problem. If one begins treatment of a severely hypothyroid (cretin) child at, let us say, 10 years of age, who has never had a dose of thyroid (fig. 9) he can predict a growth (linear increment) of from 6 to 9 inches in the first year (600–900% of the previous annual increment and 200–400% over the normal for the age) following the administration of no more than 1 to 1.5 grains of desiccated thyroid a day. During the second year the curve falls off anywhere from 25 to 50 per cent of the previous annual increment even when two or

Fig. 10. (*Read clockwise.*) ORIGINAL CARPAL DEVELOPMENT OF HYPOTHYROID CHILD C.R. at 10 yr. of age at 12 o'clock. This is approximately a 2 or 3 year development. The other carpal roentgenograms were taken at 6, 9, 12 and 18 months after the beginning of treatment.

three times the original thyroid dosage is administered. Large doses of vitamins, calcium, anterior pituitary extracts or anything we have tried fail to stimulate growth to anywhere near the previous phenomenal increment. The third year the patient will only grow at the normal or approximately normal rate irrespective of treatment (17) (it being understood, of course, that the basic daily dose of 1 to 1.5 grains of thyroid, has been continued.

What is this something which builds up in the body ready to be released by the active principle of the thyroid? Is it an accumulation of anterior pituitary growth factors stored in the blood and/or tissues or is it an enzyme or synergist which cannot exert its full influence without thyroxin? (18). Or still further, is it merely the absence of an anti-hormone which failed to accumulate during the thyroidless period and gradually does build up and put the brake upon growth when certain body concentrations have been reached? Farquharson's phenomenon (19), that is, the putting of the normal thyroid gland to rest by thyroid administration, may fit in with the latter theory. Certain it is that the thyroid hormone *per se* does not produce the growth. The administration of thyroid to normal or near normal or constitutionally inferior (but apparently not hypothyroid children) enhances the normal increment only moderately (20). The difficulties of such an observation are the natural variables in

normal growth to be encountered. Children with longstanding hyperthyroidism and with adolescent goiter are frequently taller and more advanced in osseous development than their normal companions (21) but here again the somatic change is often so slight as to be overlooked. This ability to grow and to differentiate much more rapidly than the normal for the age (when thyroid is administered to a thyroidless child) appears to increase with the years up to a given period (somewhere between 10 and 15 years of age) and then gradually subsides. The ability to change somatically never does entirely disappear, however, and some interesting experiences are encountered in the treatment of older cretins or adults with childhood myxedema who have never had the benefits of thyroid (15–16). One such case of ours is shown in figure 9.

Hypopituitary dwarfs do not respond in this manner when treated. One has to almost pull growth out of them by the administration of large doses of the material and then it is usually something less than the normal increment (22, 23, 24). I realize that this may be due to the relative impotence of anterior pituitary growth extracts available for clinical use. When the hypopituitary child (at least one who has been so diagnosed) also presents a degree of secondary hypothyroidism, growth may be enhanced by the concomitant administration of thyroid but not to a startling degree. On the other hand, we have children who present unmistakable evidence of hypothyroidism and what is also considered to be hypophyseal infantilism who do not respond to thyroid or anterior pituitary extract alone or in any combination (fig. 3). I consider such children true pituitary dwarfs. Since thyroid will not stimulate the growth of an hypophysectomized animal and we have no potent anterior pituitary growth hormone for clinical use one should not be surprised at the failure.

Some controversy seems to exist between Flower and Evans (25), Smith (12), and Evans, Simpson and Pencharz (18) on the one hand and Salmon (26) on the other as to the effect of anterior pituitary extracts and pituitary transplants on thyroidectomized rats. The former have been able to produce growth in excess of the normal by such an approach (although not so readily as when the thyroid is intact) while the latter has found pituitary transplants and anterior pituitary extracts of no value in restoring growth when the animals have been thyroidectomized at birth. We certainly were unable to influence either growth or osseous unfolding by the administration of anterior pituitary extract for a year to a classic cretin child of 10 who had received no thyroid since birth (17). Our failure may have been due to impotent extracts or underdosage but Salmon's findings put a different light on the subject. Thyroxin is stored in the tissues after a few days or weeks of life and is eliminated very slowly after the thyroid is removed. At least we know it takes from 3 to 6 months for a patient to develop myxedema after radical thyroidectomy (27) and some never do present symptoms of hypothyroidism. I have also seen patients who previously had frank myxedema go long periods after thyroid withdrawal before the hypothyroid symptoms re-established themselves. Is it then not possible that those observers able to produce excessive growth by anterior pituitary extracts owe their success to some concomitant stored or accessory thyroid effect and that the thyroid hormone after all is essential to the function of the growth fraction of the anterior pituitary?

There seems to be little doubt, in experimental animals at least, that anti-hormones can be and are developed by prolonged administration of anterior lobe extracts (28–29). Such a bugbear has never deterred our clinical investigations, however, and I satisfied myself long ago both clinically and experimentally (30) that with the extracts available there was little danger of producing such a phenomenon. Also the

children treated ten years ago with fairly large doses of extract (four to thirteen cc. daily of Evans' early crude extract) over periods ranging from one to several years, failed to show any greater degree of pituitary embarrassment after the treatment period than before. Several who were obviously not pituitary dwarfs but only constitutionally short children went into adolescence normally, received their growth spurts late, and wound up as perfectly normal but short men and women which they probably would have been anyway. The same also may be said about the concern of closing the epiphyses too early. While we know anterior pituitary extracts do effect epiphyseal growth (31–32), and no doubt closure, I have never seen premature closure except perhaps in one treated child. These effects or dearth of effects may all be due to the impotence of the material available and as a consequence the relative inadequate dosage employed in clinical practice.

Evans and his associates (33) strongly contend that the growth-stimulating factor of the anterior pituitary rests in a single active principle and have been able to develop a more potent growth producing extract which is almost, if not entirely, devoid of other activity (thyrotropic and lactogenic) Riddle (34) on the other hand still feels that the ability to stimulate growth may rest in an interplay of anterior pituitary principles rather than in a single hormone. At least, he feels there is still no conclusive proof of the specificity of a growth hormone. Clinically we are not concerned with this but I would be much happier if the extracts would do something—anything—including stimulate growth in children. As it is, we abandoned its use several years ago except in certain experimental problems. In spite of my early enthusiasm I have seen but one proven case of growth stimulation in a child by the use of such material (fig. 1). This child had absolutely nothing but the anterior pituitary extract in large doses for several years. Every other patient I have seen recorded as treated successfully received thyroid and/or vitamins and minerals, HCl or some other medicament which may have influenced growth concomitantly.

One can deal briefly with what is known concerning the influence of the active principles of other glands upon growth and development. Dorff (35) used chorionic gonadotropin in one of identical twins and in other patients (36) successfully. Buchanan and Bellweg (37) have reported similar effects upon somatic growth and the growth mechanism of adolescence through influence upon the gonad in an artificial manner. In 1930 and 1931 when anterior pituitary fractions and gonadotropins as well as our clinical experiences were young, I was treating a child for marked understature with a crude extract of the anterior pituitary. He had severe genital hypoplasia so I also gave him a daily dose of the first aqueous extract of chorionic gonadotropin (Antuitrin S). Much to my surprise he developed genitally very rapidly and also grew at a rapid rate which I, of course, erroneously attributed to the growth hormone. Unfortunately, after the initial spurt his epiphyses closed and he remains a short adult (fig. 7). At that he may have fulfilled the destiny of his germ plasm as his parents were short. This combination of the anterior pituitary (synergist) and chorionic gonadotropin is now on the market under the name of Synapoidin.

More directly, methyl testosterone and testosterone propionate have been administered to experimental animals (38–39) and to children, (40, 41, 42) *a*), to make them grow; *b*), to stop them from growing. Both apparently have been successful, although some report negative results in one direction or another. This seeming paradox is a normal phenomenon. The administration of the sex hormones, at least the male sex hormones, as well as the chorionic gonadotropin stimulates growth through the same mechanism as adolescence. It is also known that following the growth spurt of normal adolescence the growth curve drops off sharply and ultimately ceases al-

together. I feel that while selected cases of infantilism may profit by the careful administration of such preparations one should ask himself in every case if nature would not give the patient an equal or even better completed somatic set-up if left to her own devices. Certainly nature is remiss many times and occasionally very tardy but I have seen untreated children grow as much as 6 inches between 15 and 20 years of age.

Shay et al (39) feel that while the estrogens inhibit the growth hormone, testosterone stimulates its production. This I feel remains to be verified at least in the human being. One must not forget the first and most ancient lesson in clinical endocrinology, namely, that capons are larger than cocks, steers are heavier than bullocks and eunuchs (castrated early) are taller than normal men. From these early experiments it would appear that while the gonad stimulates growth for a brief season during adolescence, it acts as a brake on growth in an even greater capacity at a later period of life.

To further confuse (perhaps ultimately to elucidate) the picture Varney, Kenyon and Koch (43) have recently presented a group of girls with an association of short stature, retarded sexual development and high urinary gonadotropin, which they term "ovarian dwarfism." They kindly give me credit for having observed and spoken of such a phenomenon several years ago. But even after reading their excellent paper I am still unconvinced that the primary growth difficulty does not lie with a selective action of the pituitary rather than with the ovary *per se*. One patient of ours with congenital absence of both ovaries was tall and slender and structurally, at least, resembled the male hypogonad.

Several observers have demonstrated a depressing effect of the adrenal cortex on somatic growth (45–48) but so far as I know it has no clinical significance except perhaps in macrogenitosomia of adrenal cortex origin or in certain cases of early adrenogenital syndrome. Children so afflicted get the growth spurt in early life and as a consequence are much larger than their companions of the same age during juvenility. The epiphyses of the afflicted child tend to mature and close early, however, while those of the normal remain open until after adolescence. The normal child, therefore, has a longer growth period and should develop into a taller individual. The same may be said, with caution, of the child who is precipitated into macrogenitosomia and premature adolescence by secreting tumors of the gonads. The effect of early estrin elaboration upon epiphyseal closure time is apparent in the following case. I was fortunate in securing the present height and weight of Bennett's case of pubertas praecox (44) which is 11 years after the case was originally observed and 10 years after it was reported (Fig. 8). The patient, now 14, is 55.5 inches in height and weighs 100 pounds. The average normal female at 14 would be somewhere between 59.3 inches and 63.5 inches in height. While no recent roentgenograms are available it is presumed that the child's epiphyses either have closed or are in the process of closing. It predicates a poor somatic end result. The mother and father were of normal stature. Bennett felt that the child probably had a secreting tumor of the ovary but operation, even adequate examination, was refused.

The effect of the internal secretion of the pancreas upon growth is probably only a secondary or nutritional one. Diabetic children of long standing are stunted, but if treated early enough they resume growth at a normal rate. So far as the effect of the thymus on growth is concerned what began as a spectacular promise ends in complete disappointment (49, 50). The secretory status of the parathyroid glands probably influences the stature of the child through control of mineral balance. Usually, however, disturbances of the parathyroid are only objectively evidenced in distortion

of the skeleton and not in dwarfing as we think of it. The last sentence brings us again to the initial point that growth is a very complex phenomenon, especially in primates, and that no secreting organ of the body may be entirely exempt from exerting an influence in its production.

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CHANGING CONCEPTS IN THE TREATMENT OF TOXIC GOITER

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THE TREATMENT of toxic goiter has undergone a gradual evolution which is concerned primarily with ways and means of reducing the mortality from thyroidectomy, because subtotal thyroidectomy after adequate preparation remains the treatment of choice. The internist, the physiologist and the biochemist interested in the nature of the disease feel that eventually a non-surgical method of treatment may be found when we know its cause and are able to attack it. There have been some recent interesting developments along this line which involve an attempt to suppress the thyrotropic activity of the pituitary and the control of the thyroid itself by the use of radioactive iodine. Both procedures are still in the experimental stage. The use of the roentgen ray has been largely relegated to the treatment of occasional patients who can not be prepared for operation with iodine, rest and a high caloric diet. A few patients who have the disease in mild form are still treated by iodine alone, but this method is fraught with danger because a mild case may at any time become a severe one and thus increase the risk of operation. The evolution of our present ideas of treatment naturally divides itself into two periods, *a*), before iodine was used and, *b*), after iodine.

Treatment of Toxic Goiter Before Iodine was Used.

The period before iodine therapy was characterized by the gradual development of the stage operation and the introduction of irradiation of the thyroid by the roentgen ray or by radium. It is not generally appreciated how greatly the mortality had been reduced before the introduction of iodine (1). In leading goiter clinics it had dropped to between 1 and 4 per cent, although the mortality for the country as a whole was much higher than this—perhaps from 10 to 15 per cent. Since the introduction of iodine it has dropped to between 0.25 and 1 per cent in leading clinics. Of the greatest interest is the gradual reduction in mortality which was reported not only among surgeons in general but in the experience of individual surgeons. Thus from 1894 to 1907, according to the table in Crotti's (2) book, it varied from 2.85 to 30.0 per cent, with a mortality of over 10 per cent in 10 of 23 reports. From 1908 to 1917 it varied for the most part from 0 to 13.1 per cent and it was over 5 per cent in only 7 of 28 reports. Unusually high rates were reported by some outstanding surgeons in their first few thyroidectomies. Thus the Mayos (3–12) had 4 deaths in their first 16 operations, 3 in their next 30 and 3 in the next 150. From 1907 to 1920 their mortality varied from 2 to 5 per cent with a general trend downward and it was for the most part under 3 per cent after 1912. Similar experiences were recorded by Crile (13, 14), Lahey (15, 16), Porter (17), Kocher (18), Riedl (19), Crotti (2) and others. All of these men had reduced their mortality rate to less than 4 per cent before the introduction of iodine and several of them to as low as 1 per cent. This reduction

was commonly attributed to the development of the stage operation but Richter (20), whose mortality was as low as 1 per cent, attributed his successful results to the performance of the operation in one stage. Our experience of the last 17 years in the treatment of toxic goiter leads us to question the notion of the importance of the stage operation in reducing mortality. We are inclined to think that the development of good surgical judgment played a very important rôle. With experience, surgeons improved their technique, thereby causing less trauma and fewer complications. They learned when patients would tolerate surgical procedures and when they would not. They learned that loss of weight, great emotional instability and muscle weakness were unfavorable signs. An appreciation of the value of rest and of the removal of psychic traumata gradually developed. In some quarters attention was paid to increasing the caloric intake by special diets but in general this phase of the subject did not receive the attention it deserved. We suspect that the mortality might have been reduced to just as low a figure if the operation had been carried out in only one stage in most instances but greater attention paid to the preoperative preparation of the patient.

The stage operation was time consuming, expensive and unpleasant for the patient. The actual procedure varied in different clinics. In severe cases it was common for the patient to have: *a*), a polar ligation; *b*), in 4 to 10 days, another polar ligation; *c*), in 6 to 8 weeks, a hemithyroidectomy; and *d*), in another 2 months, a second hemithyroidectomy.

Because the surgical treatment was so long drawn out and the mortality so high in the hands of the average surgeon, the use of irradiation of the thyroid was developed in some clinics. This form of therapy had the advantage of being relatively safe but it was effective in reducing the basal metabolism permanently to within normal limits in only about one-third of the patients (21).

After Iodine Therapy

The use of iodine therapy in toxic goiter was introduced in 1922 by Plummer (22). The period since iodine has been characterized by: *a*), the elimination of the stage operation, *b*), its later revival in some clinics because of too great reliance upon iodine, *c*), renewed appreciation of the value of factors other than iodine in the management of the disease.

Iodine, by reducing the intensity of the disease, has diminished the risk of operation, but following its introduction it was relied upon too much and other factors known to have a favorable influence on the course of the disease were disregarded. Although it was supposed to abolish postoperative crises, it soon became apparent that patients were still dying from this cause. As a result, the stage operation was revived in some clinics. Clute (23) in summarizing the experiences of the Lahey Clinic, stated "With multiple stage operations the mortality of primary hyperthyroidism was reduced to less than 1 per cent in this clinic and we hesitated to adopt the more radical procedure of thyroidectomy in one stage, which was popular after iodine preparation. In 1925, however, we became more radical and, to a large extent, we permitted iodine to replace pole ligations. The number of stage operations fell from 65 per cent, the figure before iodine was used, to 21 per cent. The mortality rose at once to 2.06 per cent . . . ."

In our experience we have gradually almost eliminated the stage operation by substituting for it more adequate preoperative preparation of the patient. We almost never permit a thyroidectomy to be performed unless we are fairly certain that the patient will tolerate the operation well. We had an unusual opportunity to study the mortality from thyroidectomy in the Cook County Hospital where we followed about

half of the patients and therefore had an adequate control series (24, 25, 26). By great attention to the preoperative and postoperative care of the patients we were able to reduce the mortality from about 10 per cent to about 1.5 per cent. We were forced to the conclusion that the single most important factor in bringing about this reduction in mortality was improvement in the preoperative care of the patient. The surgeons and the surgical technique remained essentially the same. Thus, during the period of 1932 to 1937, three of the best surgeons had 5 deaths in performing 222 thyroidectomies for Thompson and Taylor—a mortality of 2.3 per cent, whereas they had 22 deaths in performing 297 thyroidectomies for other medical men—a mortality of 7.4 per cent.

So great is the importance of preoperative care that we have come to the conclusion that a crisis in the postoperative period usually means inadequate preoperative preparation. A thyroidectomy is not an emergency operation and should not be carried out unless the condition of the patient warrants it.

We have discussed in detail elsewhere the various factors that are important in the preoperative preparation of patients (24, 27, 28, 29, 30, 31). These include the following points.

1. A diet sufficiently high in calories to produce a gain in weight.
2. The administration of iodine.
3. Rest.
4. Roentgen ray treatment in patients with high metabolic rates refractory to iodine.
5. The administration of digitalis in cardiac decompensation.

In the immediate preoperative period the following procedures are important:

1. To make a careful search for upper respiratory infections or a sudden increase in the severity of the disease.
2. To administer a carbohydrate meal from 6 to 8 hours before operation.
3. To administer the regular dose of iodine with this meal.
4. To institute, at least 24 hours before the scheduled time of operation, some program suitable for control of emergencies in patients in whom the disease is complicated by diabetes mellitus.

Our ideas concerning these factors have undergone a gradual evolution. We have come to regard gain in weight as the single most important factor in determining the outcome of operation. With gain in weight there develops reduction in emotional instability and in muscle weakness, which Plummer and others made so much of in determining the risk of operation. If a patient can eat well it is almost always possible to administer enough calories to produce a gain in weight: and if the patient can not eat well, the risk of operation is great.

Our ideas about iodine have changed. It used to be taught that operative procedures should be carried out as soon as a maximum reduction in metabolism was noted following the institution of treatment with iodine, namely, in about 10 days. It was felt that if treatment were continued beyond this point there was great danger of developing refractoriness to iodine with resultant increase in the risk of operation. At present we worry very little about the development of refractoriness even though it may occur in rare instances. We almost never perform a thyroidectomy as soon as the maximum reduction in metabolism takes place but nearly always wait for 7 to 10 days longer in order to allow the patient time to derive the benefit from the lowered rate of metabolism. In some instances operation is delayed for several months. If the metabolism rises slightly it is no cause for concern. If the metabolism fails to show any reduction during the administration of iodine it may usually be disregarded,

provided the patient is gaining weight steadily. The reaction to iodine which troubles us is a rapid increase in metabolism during its administration. This phenomenon does not occur very often and when it does, roentgen ray therapy should be instituted at once. Whether or not iodine should be discontinued is a debatable point. We have actually observed the development of refractoriness to iodine and have seen recovery from this refractoriness following the omission of iodine. It can not be said, however, that the omission of iodine caused the disappearance of the refractoriness. It may have been the result of the natural course of the disease.

We have been interested in determining the range of effective iodine dosage in exophthalmic goiter (32, 33). In general, it may be said that from the standpoint of routine therapy, the size of the dose does not matter within very wide limits and the form in which iodine is given is not of much importance. It continues to be most commonly used in the form of the compound solution and it is probably true that the daily administration of 5 minims of this solution (40 mg. of iodine) will always produce a maximum effect. In routine hospital practice we use 5 minims two or three times daily.

Our ideas about rest have been modified. While confinement to the hospital is very important in the preoperative preparation, complete bed rest appears to be harmful except in the presence of a crisis or cardiac decompensation. It causes loss of muscle tone, reduces the appetite and makes patients unnecessarily nervous.

The indications for thyroidectomy in the presence of heart disease are the same as in its absence, except when decompensation ensues. Toxic goiter rarely causes an undamaged heart to fail and the development of auricular fibrillation and decompensation are evidence that the heart is independently damaged. In the presence of one or both, the administration of digitalis is indicated but it plays no rôle in the routine management of toxic goiter.

By paying great attention to the preoperative care of patients and withholding surgical procedures until it is reasonably certain that the patients can tolerate them, we have virtually eliminated postoperative crises. Practically all of the complications which we now see in the postoperative period are surgical in nature. Important among these are pneumonia and sudden respiratory difficulty commonly associated with hemorrhage and formation of clots under the strap muscles of the neck. Deaths from pneumonia may now be greatly reduced by the employment of chemotherapy and most deaths from sudden respiratory difficulty can be prevented by careful observation of the patient to detect bleeding and by having trained personnel immediately available for the passage of a life-saving tube and the performance of a tracheotomy.

Our experience leads us to believe that the necessity for stage operations may be avoided in most instances by producing maximum improvement before operation. Whether the patient will live or die is determined largely by the type of preoperative care employed. In particular, we should like to point out that the ligation of arteries has been virtually discarded. Its only value would appear to be to test the ability of the patient to withstand surgical procedures when there is any doubt about it and this can usually be determined in other ways. We do not wish to minimize the importance of surgical skill. We think it is very important but not as much so as preoperative care.

Recent Developments in Non-surgical Procedures

A few years ago a thyrotropic factor was isolated from the pituitary gland which produced many interesting effects. In animals its administration was followed by

changes resembling those seen in toxic goiter. In man it produced exacerbation of toxic goiter, made patients with non-toxic goiter temporarily thyrotoxic, and in patients with normal thyroid function, produced a syndrome resembling exophthalmic goiter except that definite exophthalmos was not observed (34, 35). In both man and animals it was noted that 'immunity' developed after a few weeks. In spite of continued treatment, the metabolism would drop to its original level or a lower one. In animals thus treated the serum contained a substance which prevented the rise in metabolism when the thyrotropic factor was administered to previously untreated animals. In occasional patients with exophthalmic goiter, the metabolism, after an initial rise, would fall to a lower level than that observed before treatment and in some instances to within normal limits (35). An attempt has been made to treat patients with exophthalmic goiter with the serum of horses immunized to the thyrotropic factor but without very satisfactory results. Irradiation of the pituitary has been employed but the results are uncertain.

Hamilton and Lawrence (36) and Hertz and Roberts (37) have had some success experimentally in destroying the thyroid by the administration of radioactive iodine. This observation has interesting possibilities in the treatment of toxic goiter. When a small dose of iodine is administered, most of it is retained by the thyroid and the damage is therefore limited to this organ. These observations are still in the experimental stage.

SUMMARY

The evolution of the present day treatment of exophthalmic goiter has been presented. The treatment of choice is still subtotal thyroidectomy performed by a well trained surgeon after very careful preoperative preparation of the patient. Although iodine is valuable, too much reliance should not be placed on it. Its administration should be supplemented with other forms of therapy known to be beneficial. The most important of these is the administration of a high caloric diet. Gain in weight is the single most favorable sign in determining the outcome of operation.

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